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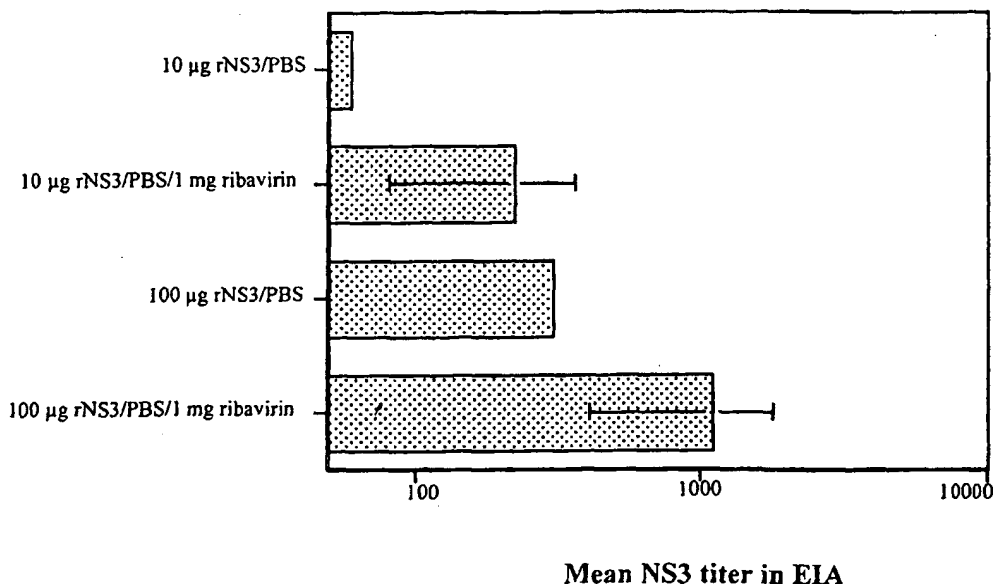
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(54) Title: VACCINES CONTAINING RIBAVIRIN AND METHODS OF USE THEREOF



(57) Abstract: Compositions and methods for enhancing the effect of vaccines in animals, such as domestic, sport, or pet species, and humans are disclosed. More particularly, vaccine compositions comprising ribavirin and an antigen, preferably an antigen that has an epitope present in Hepatitis C virus (HCV), are disclosed for use in treating and preventing disease, preferably HCV infection.

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VACCINES CONTAINING RIBAVIRIN AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

The present invention relates to compositions and methods for enhancing the effect of
5 vaccines in animals, such as domestic, sport, or pet species, and humans. More particularly,
preferred embodiments concern the use of ribavirin as an adjuvant and compositions having
ribavirin and an antigen.

BACKGROUND OF THE INVENTION

The use of vaccines to prevent disease in humans, farm livestock, sports animals, and
10 household pets is a common practice. Frequently, however, the antigen used in a vaccine is not
sufficiently immunogenic to raise the antibody titre to levels that are sufficient to provide
protection against subsequent challenge or to maintain the potential for mounting these levels over
extended time periods. Further, many vaccines are altogether deficient in inducing cell-mediated
immunity, which is a primary immune defense against bacterial and viral infection. A
15 considerable amount of research is currently focussed on the development of more potent vaccines
and ways to enhance the immunogenicity of antigen-containing preparations. (See e.g., U.S. Pat.
Nos. 6,056,961; 6,060,068; 6,063,380; and Li et al., *Science* 288:2219-2222 (2000)).

Notorious among such "weak" vaccines are hepatitis B vaccines. For example,
recombinant vaccines against hepatitis B virus such as Genhevacb (Pasteur Merieux Serums et
20 Vaccines, 58, Avenue Leclerc 69007 Lyon, France), Engerixb (Smith, Kline and Symbol French),
and Recombivaxhb (Merck, Sharp, and Dhome) are effective only after at least three injections at
0, 30, and 60 or 180 days, followed by an obligatory booster after one year. (Chedid et al., U.S.
Patent No. 6,063,380). Additionally, many subjects receiving these vaccines respond poorly, if at
all. Because many regions of the world are endemic for HBV infection, the poorly immunogenic
25 character of existing HBV vaccines has become an extremely serious problem.

To obtain a stronger, humoral and/or cellular response, it is common to administer a
vaccine in a material that enhances the immune response of the patient to the antigen present in the
vaccine. The most commonly used adjuvants for vaccine protocols are oil preparations and alum.
(Chedid et al., U.S. Patent No. 6,063,380). A greater repertoire of safe and effective adjuvants is
30 needed.

Nucleoside analogs have been widely used in anti-viral therapies due to their capacity to
reduce viral replication. (Hosoya et al., *J. Inf. Dis.*, 168:641-646 (1993)). ribavirin (1- β -D-
ribofuranosyl-1,2,4-triazole-3-carboxamide) is a synthetic guanosine analog that has been used to
inhibit RNA and DNA virus replication. (Huffman et al., *Antimicrob. Agents. Chemother.*, 3:235
35 (1973); Sidwell et al., *Science*, 177:705 (1972)). ribavirin has been shown to be a competitive

inhibitor of inositol mono-phosphate (IMP) dehydrogenase (IMPDH), which converts IMP to IMX (which is then converted to GMP). De Clercq, Anti viral Agents: characteristic activity spectrum depending on the molecular target with which they interact, Academic press, Inc., New York N.Y., pp. 1-55 (1993). Intracellular pools of GTP become depleted as a result of long term ribavirin treatment.

In addition to antiviral activity, investigators have observed that some guanosine analogs have an effect on the immune system. (U.S. Patent Nos. 6,063,772 and 4,950,647). ribavirin has been shown to inhibit functional humoral immune responses (Peavy et al., *J. Immunol.*, 126:861-864 (1981); Powers et al., *Antimicrob. Agents. Chemother.*, 22:108-114 (1982)) and IgE-mediated modulation of mast cell secretion. (Marquardt et al., *J. Pharmacol. Exp. Therapeutics*, 240:145-149 (1987)). Some investigators report that a daily oral therapy of ribavirin has an immune modulating effect on humans and mice. (Hultgren et al., *J. Gen. Virol.*, 79:2381-2391 (1998) and Cramp et al., *Gastron. Enterol.*, 118:346-355 (2000)). Nevertheless, the current understanding of the effects of ribavirin on the immune system is in its infancy.

SUMMARY OF THE INVENTION

It has been discovered that ribavirin can be used as an adjuvant to enhance or facilitate an immune response to an antigen. Embodiments of the invention described herein include "strong" vaccine preparations that comprise an antigen and ribavirin. Generally, these preparations have an amount of ribavirin that is sufficient to enhance or facilitate an immune response to the antigen. Other aspects of the invention include methods of enhancing or facilitating an immune response of an animal, including a human, to an antigen. By one approach, for example, an animal in need of a potent immune response to an antigen is identified and then is provided an amount of ribavirin together with the antigen. In some methods, the ribavirin and the antigen are provided in combination (e.g., in a single composition) and in others, the ribavirin and the antigen are provided separately. Several embodiments also concern the manufacture and use of compositions having ribavirin and an antigen.

Although the embodied compositions include ribavirin and virtually any antigen or epitope, preferred compositions comprise ribavirin and a hepatitis viral antigen or epitope. The antigen or epitope can be peptide or nucleic acid-based (e.g., a RNA encoding a peptide antigen or a construct that expresses a peptide antigen when introduced to a subject). Compositions having ribavirin and a peptide comprising an antigen or epitope from the hepatitis A virus (HAV) or a nucleic acid encoding said peptide are embodiments. Compositions having ribavirin and a peptide comprising an antigen or epitope from the hepatitis B virus (HBV) or a nucleic acid encoding said peptide are embodiments. HBV antigens that are suitable include, for example, hepatitis B surface antigen (HBsAg), hepatitis core antigen (HBcAg), hepatitis e antigen (HBeAg), and nucleic acids

encoding these molecules. Still further, compositions having ribavirin and a peptide comprising an antigen or epitope from the hepatitis C virus (HCV) or a nucleic acid encoding said peptide are embodiments. Suitable HCV antigens include, but are not limited to, one or more domains of the HCV sequence (e.g., NS3 and/or NS4A) and nucleic acids encoding said molecules.

5 A new HCV sequence was also discovered. A novel NS3/4A fragment of the HCV genome was cloned and sequenced from a patient infected with HCV (SEQ. ID. NO.: 16). This sequence was found to be only 93% homologous to the most closely related HCV sequence. This novel peptide (SEQ. ID. NO.: 17) and fragments thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length, nucleic acids encoding these molecules, vectors having said nucleic acids, and cells
10 having said vectors, nucleic acids, or peptides are also embodiments of the present invention. A particularly preferred embodiment is a vaccine composition comprising ribavirin and the HCV peptide of SEQ. ID. NO.: 17 or a fragment thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NO.: 25) or a nucleic acid encoding said peptide or fragments.

Additionally, it was discovered that truncated mutants and mutants of the NS3/4A peptide,
15 which lack a proteolytic cleavage site, are highly immunogenic. These novel peptides (SEQ. ID. NOs.: 29- 32 and 43-49) and fragments thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 26, 27, and 33-42), nucleic acids encoding these molecules, vectors having said nucleic acids, and cells having said vectors, nucleic acids, or peptides are also embodiments. A particularly preferred embodiment is a vaccine composition comprising ribavirin
20 and at least one HCV peptide of SEQ. ID. NOs.: 29- 32 and 43-49 or a fragment thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 26, 27, and 33-42) or a nucleic acid encoding said peptides or fragments.

Furthermore, compositions having a mixture of the antigens above are embodiments of the invention. For example, some compositions comprise a HBV antigen, a HAV antigen, and
25 ribavirin or a HBV antigen, a HCV antigen, and ribavirin or a HAV antigen, a HCV antigen, and ribavirin or a HBV antigen, a HAV antigen, a HCV antigen, and ribavirin. Other embodiments comprise ribavirin and a nucleic acid encoding a mixture of the antigens described above. Some embodiments also include other adjuvants, binders, emulsifiers, carriers, and fillers, as known in the art, including, but not limited to, alum, oil, and other compounds that enhance an immune
30 response.

Methods of making and using the compositions described herein are also aspects of the invention. Some methods are practiced by mixing ribavirin with a peptide or nucleic acid antigen (e.g., an HAV, HBV, HCV antigen) so as to formulate a single composition (e.g., a vaccine composition). Preferred methods involve the mixing of ribavirin with an HCV antigen that has an
35 epitope present on one or more domains of HCV (e.g., NS3 and/or NS4A).

Preferred methods of using the compositions described herein involve providing an animal in need with a sufficient amount of ribavirin and a hepatitis viral antigen (e.g., HBV antigen, HAV antigen, HCV antigen a nucleic acid encoding one of these antigens or any combination thereof). By one approach, for example, an animal in need of potent immune response to a hepatitis viral antigen (e.g., an animal at risk or already infected with a hepatitis infection) is identified and said animal is provided an amount of ribavirin and a hepatitis viral antigen (either in a single composition or separately) that is effective to enhance or facilitate an immune response to the hepatitis viral antigen. Preferably, an animal in need of a potent immune response to HCV is identified and said animal is provided a composition comprising ribavirin and a peptide comprising an antigen or epitope present on SEQ. ID. NO.: 1, 6, 7, or 17 or a nucleic acid encoding said peptide. Particularly preferred methods involve the identification of an animal in need of an potent immune response to HCV and providing said animal a composition comprising ribavirin and an amount of an HCV antigen (e.g., NS3/4A (SEQ. ID. NO.: 17), mutant NS3/4A SEQ. ID. NOs.: 29- 32 and 43-49, or a fragment thereof at least 3, 4-10, 10-20, 20-30, or 30-50 amino acids in length (e.g., SEQ. ID. NOs.: 25-27, and 33-42) or a nucleic acid encoding one or more of these molecules) that is sufficient to enhance or facilitate an immune response to said antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a graph showing the humoral response to 10 and 100µg recombinant Hepatitis C virus (HCV) non structural 3 protein (NS3), as determined by mean end point titres, when a single dose of 1mg of ribavirin was co-administered.

FIGURE 2 is a graph showing the humoral response to 20µg recombinant Hepatitis C virus (HCV) non structural 3 protein (NS3), as determined by mean end point titres, when a single dose of 0.1, 1.0, or 10mg of ribavirin was co-administered.

FIGURE 3 is a graph showing the effects of a single dose of 1mg ribavirin on NS3-specific lymph node proliferative responses, as determined by *in vitro* recall responses.

FIGURE 4 is a graph showing the antibody titer in H-2^d mice against NS3 as a function of time after the first immunization. Diamonds denote antibody titer in mice immunized with NS3/4A-pVAX and squares denote antibody titer in mice immunized with NS3-pVAX.

FIGURE 5A is a graph showing the percentage of specific CTL-mediated lysis of SP2/0 target cells as a function of the effector to target ratio. Phosphate Buffered Saline (PBS) was used as a control immunogen.

FIGURE 5B Is a graph showing the percentage specific CTL-mediated lysis of SP2/0 target cells as a function of the effector to target ratio. Plasmid NS3/4A-pVAX was used as the immunogen.

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that compositions comprising ribavirin and an antigen (e.g., a molecule containing an epitope of a pathogen such as a virus, bacteria, mold, yeast, or parasite) enhance and/or facilitate an animal's immune response to the antigen. That is, it was discovered that ribavirin is an effective "adjuvant," which for the purposes of this disclosure, refers to a material that has the ability to enhance or facilitate an immune response to a particular antigen. The adjuvant activity of ribavirin was manifested by a significant increase in immune-mediated protection against the antigen, an increase in the titer of antibody raised to the antigen, and an increase in proliferative T cell responses.

Several compositions (e.g., vaccines) that comprise ribavirin and an antigen or epitope are described herein. Vaccine formulations containing ribavirin, for example, can vary according to the amount of ribavirin, the form of ribavirin, and the type of antigen. The antigen can be a peptide or a nucleic acid (e.g., a RNA encoding a peptide antigen or a construct that expresses a peptide antigen when introduced into a subject). Preferred compositions comprise ribavirin and a hepatitis viral antigen (e.g., HAV antigen, HBV antigen, HCV antigen, a nucleic acid encoding these molecules, or any combination thereof). In particular, at least one HCV antigen or an epitope present on SEQ. ID. NO.: 1 or a nucleic acid encoding said HCV antigen are desired for mixing with ribavirin to make said compositions. That is, some embodiments include, but are not limited to, compositions comprising ribavirin and a peptide comprising SEQ. ID. NO.: 1, or a fragment thereof having at least 2500, 2000, 1600, 1200, 800, 400, 200, 100, 50, 10, or 3 consecutive amino acids of SEQ. ID. NO.: 1. Additional embodiments concern compositions comprising ribavirin and a nucleic acid encoding SEQ. ID. NO.: 13 or a fragment thereof having at least 9, 12, 15, 20, 30, 50, 75, 100, 200, 500 consecutive nucleotides of SEQ. ID. NO.: 13.

Other embodiments include a composition (e.g., a vaccine) that comprises ribavirin and a specific fragment of SEQ. ID. NO.: 1, wherein said fragment corresponds to a particular domain of HCV. Some embodiments, for example, comprise a fragment of HCV corresponding to amino acids 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 of SEQ. ID. NO.: 1. Compositions comprising ribavirin and a nucleic acid encoding one or more of these fragments are also embodiments of the invention.

Additionally, a novel HCV sequence was discovered. A novel nucleic acid and protein corresponding to the NS3/4A domain of HCV was cloned from a patient infected with HCV (SEQ. ID. NO.: 16). A Genebank search revealed that the cloned sequence had the greatest homology to HCV sequences but was only 93% homologous to the closest HCV relative (accession no AJ 278830). This novel peptide (SEQ. ID. NO.: 17) and fragments thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length, nucleic acids encoding these molecules, vectors having said nucleic

acids, and cells having said vectors, nucleic acids, or peptides are also embodiments of the invention. Further, some of the vaccine embodiments described herein comprise ribavirin and this novel NS3/4A peptide or a fragment thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NO.: 25) or a nucleic acid encoding one or more of these molecules.

5 Mutants of the novel NS3/4A peptide were also created. It was discovered that truncated mutants (e.g., SEQ. ID. NO.: 29) and mutants, which lack a proteolytic cleavage site, are highly immunogenic. These novel peptides SEQ. ID. NOs.: 29- 32 and 43-49 and fragments thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 26, 27, and 33-42), nucleic acids encoding these molecules, vectors having said nucleic acids, and cells having said
10 vectors, nucleic acids, or peptides are also embodiments. Furthermore, some of the compositions described herein comprise ribavirin and at least one of the mutant HCV peptides described above or a fragment thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length. Other vaccine embodiments comprise ribavirin and a nucleic acid (e.g., DNA) encoding one or more of the peptides described above.

15 Methods of making and using the compositions above are also embodiments. For example, the compositions described above can be made by providing ribavirin, providing an antigen (e.g., a peptide comprising an HCV antigen or a nucleic acid encoding said peptide), and mixing said ribavirin and said antigen so as to formulate a composition that can be used to enhance or facilitate an immune response in a subject to said antigen. Preferred methods entail mixing a
20 preferred antigen or epitope (e.g., a peptide comprising SEQ. ID. NO.: 1, 6, 7, or 17 or specific fragments thereof, such as amino acids 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, 1972-3011 of SEQ. ID. NO.: 1 and nucleic acids encoding these molecules) with ribavirin. Other antigens or epitopes can also be mixed with ribavirin including, but not limited to, fragments of SEQ. ID. NO.: 1 that have at least 2500, 2000, 1600, 1200, 800, 400, 200,
25 100, 50, 10, or 3 consecutive amino acids and nucleic acids encoding these fragments. Particularly preferred methods concern the making of vaccine compositions comprising the newly discovered NS3/4A fragment or an NS3/4A mutant (e.g., a truncated mutant or a mutant lacking a proteolytic cleavage site), or a fragment thereof of at least four amino acids in length or a nucleic acid encoding one or more of these molecules.

30 Methods of enhancing or facilitating the immune response of an animal, including humans, to an antigen are embodiments of the invention. Such methods can be practiced, for example, by identifying an animal in need of a potent immune response to an antigen/epitope and providing said animal a composition comprising the antigen/epitope and an amount of ribavirin that is effective to enhance or facilitate an immune response to the antigen/epitope. In some
35 embodiments, the ribavirin and the antigen are administered separately, instead of in a single

mixture. Preferably, in this instance, the ribavirin is administered a short time before or a short time after administering the antigen. Preferred methods involve providing the animal in need with ribavirin and a hepatitis antigen (e.g., HAV antigen, HBV antigen, HCV antigen, a nucleic acid encoding these molecules, or any combination thereof). Some of these methods involve HCV
 5 antigens, such as a peptide comprising SEQ. ID. NO.: 1, or a fragment thereof having at least 2500, 2000, 1600, 1200, 800, 400, 200, 100, 50, 10, or 3 consecutive amino acids of SEQ. ID. NO.: 1. Additional methods involve compositions comprising ribavirin and a nucleic acid encoding SEQ. ID. NO.: 13 or a nucleic acid encoding one or more of the fragments discussed above.

10 Some preferred methods, for example, concern the use of a composition (e.g., a vaccine) that comprises ribavirin and a peptide comprising SEQ. ID. NO.: 1 or a specific fragment thereof, which corresponds to an HCV domain including, but not limited to, a peptide comprising amino acids 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 of
 15 SEQ. ID. NO.: 1. Particularly preferred methods concern the use of a vaccine composition comprising the NS3/4A fragment of SEQ. ID. NO.: 17 or the mutant NS3/4A (e.g., SEQ. ID. NOs.: 29- 32 and 43-49), which lack a proteolytic cleavage site, or a fragment thereof of at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 26, 27, and 33-42). Compositions comprising ribavirin and a nucleic acid encoding these fragments can also be used with the methods described herein.

20 Other embodiments concern methods of treating and preventing HCV infection. By one approach, ribavirin and an HCV antigen or epitope are used to prepare a medicament for the treatment and/or prevention of HCV infection. By another approach, an individual in need of a medicament that prevents and/or treats HCV infection is identified and said individual is provided a medicament comprising ribavirin and an HCV antigen or epitope, preferably an epitope present
 25 on SEQ. ID. NO.: 1, more preferably a fragment of SEQ. ID. NO.: 1 having at least 2500, 2000, 1600, 1200, 800, 400, 200, 100, 50, 10, or 3 consecutive amino acids or most preferably a fragment of SEQ. ID. NO.: 1 such as 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 or a nucleic acid encoding SEQ. ID. NO.: 1 or said fragments above. Particularly preferred methods concern the use of a vaccine composition comprising ribavirin and
 30 the NS3/4A fragment of SEQ. ID. NO.: 17 or the mutant NS3/4A, which lacks a proteolytic cleavage site (e.g., SEQ. ID. NOs.: 29- 32 and 43-49) or a fragment thereof of at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 25-27, and 33-42) or a nucleic acid encoding one or more of these molecules. The section below discusses the use of ribavirin as an adjuvant in greater detail.

35

Ribavirin

The compositions described herein can be manufactured in accordance with conventional methods of galenic pharmacy to produce medicinal agents for administration to animals, e.g., mammals including humans. Ribavirin can be obtained from commercial suppliers (e.g., Sigma and ICN). Ribavirin and/or the antigen can be formulated into the vaccine with and without modification. For example, the ribavirin and/or antigen can be modified or derivatized to make a more stable molecule and/or a more potent adjuvant. By one approach, the stability of ribavirin and/or an antigen can be enhanced by coupling the molecules to a support such as a hydrophilic polymer (e.g., polyethylene glycol).

Many more ribavirin derivatives can be generated using conventional techniques in rational drug design and combinatorial chemistry. For example, Molecular Simulations Inc. (MSI), as well as many other suppliers, provide software that allows one of skill to build a combinatorial library of organic molecules. The C2.Analog Builder program, for example, can be integrated with MSI's suite of Cerius2 molecular diversity software to develop a library of ribavirin derivatives that can be used with the embodiments described herein. (See e.g., <http://msi.com/life/products/cerius2/index.html>).

By one approach, the chemical structure of ribavirin is recorded on a computer readable medium and is accessed by one or more modeling software application programs. The C2.Analog Builder program in conjunction with C2Diversity program allows the user to generate a very large virtual library based on the diversity of R-groups for each substituent position, for example. Compounds having the same structure as the modeled ribavirin derivatives created in the virtual library are then made using conventional chemistry or can be obtained from a commercial source.

The newly manufactured ribavirin derivatives are then screened in assays, which determine the extent of adjuvant activity of the molecule and/or the extent of its ability to modulate of an immune response. Some assays may involve virtual drug screening software, such as C2.Ludi. C2.Ludi is a software program that allows a user to explore databases of molecules (e.g., ribavirin derivatives) for their ability to interact with the active site of a protein of interest (e.g., RAC2 or another GTP binding protein). Based upon predicted interactions discovered with the virtual drug screening software, the ribavirin derivatives can be prioritized for further characterization in conventional assays that determine adjuvant activity and/or the extent of a molecule to modulate an immune response. *Example 1* describes several assays that were used to evaluate the adjuvant activity of ribavirin.

EXAMPLE 1

This following assays can be used with any ribavirin derivative or combinations of ribavirin derivatives to determine the extent of adjuvant activity of the particular composition. In a

first set of experiments, groups of three to five Balb/c mice (BK Universal, Uppsala, Sweden) were immunized *i.p* or *s.c.* (e.g., at the base of the tail) with 10µg or 100µg of recombinant hepatitis C virus non-structural 3 (rNS3) protein at weeks zero and four. The rNS3 was dissolved in phosphate buffered saline (PBS) alone or PBS containing 1mg ribavirin (obtained from ICN, Costa Mesa, CA). Mice were injected with a total volume of 100µl per injection.

At two, four, and six weeks following first *i.p.* immunization, all mice were bled by retro-orbital sampling. Serum samples were collected and analyzed for the presence of antibodies to rNS3. To determine the antibody titer, an enzyme immunoassay (EIA) was performed. (See e.g., Hultgren et al., *J Gen Virol.* 79:2381-91 (1998) and Hultgren et al., *Clin. Diagn. Lab. Immunol.* 4:630-632 (1997)). The antibody levels were recorded as the highest serum dilution giving an optical density at 405nm more than twice that of non-immunized mice.

Mice that received 10µg or 100µg rNS3 mixed with 1mg ribavirin in PBS displayed consistently higher levels of NS3 antibodies. The antibody titer that was detected by EIA at two weeks post-immunization is shown in **FIGURE 1**. The vaccine formulations having 1mg of ribavirin and either 10µg or 100µg of rNS3 induced a significantly greater antibody titer than the vaccine formulations composed of only rNS3.

In a second set of experiments, groups of eight Balb/c mice were at weeks zero and four immunized intraperitoneally with 10 or 50 µg of rNS3 in 100 µl phosphate buffered saline containing either 0 mg, 1 mg, 3 mg, or 10 mg ribavirin (Sigma). At four, six and eight weeks the mice were bled and serum was separated and frozen. After completion of the study, sera were tested for the levels of antibodies to recombinant NS3, as described above. Mean antibody levels to rNS3 were compared between the groups using Student's t-test (parametric analysis) or Mann-Whitney (non-parametric analysis) and the software package StatView 4.5 (Abacus Concepts, Berkely, CA). The adjuvant effect of ribavirin when added in three doses to 10 µg of rNS3 are provided in **TABLE 1**. The adjuvant effect of ribavirin when added in three doses to 50 µg of rNS3 are provided in **TABLE 2**. Parametrical comparison of the mean rNS3 antibody titres in mice receiving different 10µg or 50 µg of rNS3 and different doses of ribavirin are provided in **TABLES 3 and 4**, respectively. Non-parametrical comparison of mean NS3 antibody titres in mice receiving different 10µg or 50 µg of rNS3 and different doses of ribavirin are provided in **TABLES 5 and 6**, respectively. The values given represent end point titres to recombinant rNS3.

TABLE 1

Amount ribavirin (mg/dose)	Amount immunogen (µg/dose)	Mouse ID	Antibody titre to rNS3 at indicated week		
			Week 4	Week 6	Week 8
None	10	5:1	300	1500	1500
None	10	5:2	<60	7500	1500
None	10	5:3	<60	1500	300
None	10	5:4	60	1500	1500
None	10	5:5	<60	1500	nt
None	10	5:6	60	1500	1500
None	10	5:7	<60	7500	7500
None	10	5:8	300	37500	7500
Group mean titre (mean±SD)			180 ±139	7500 ±12421	3042 ±3076
1	10	6:1	300	37500	37500
1	10	6:2	<60	1500	1500
1	10	6:3	300	37500	187500
1	10	6:4	300	37500	7500
1	10	6:5	60	nt	nt
1	10	6:6	<60	37500	7500
1	10	6:7	<60	37500	7500
1	10	6:8	300	7500	7500
Group mean titre (mean±SD)			252 ±107	28071 ±16195	36642 ±67565
3	10	7:1	60	37500	7500
3	10	7:2	60	37500	37500
3	10	7:3	300	7500	7500
3	10	7:4	300	37500	7500
3	10	7:5	300	37500	37500
3	10	7:6	300	37500	37500
3	10	7:7	60	7500	7500
3	10	7:8	60	37500	37500
Group mean titre (mean±SD)			180± 128	30000± 13887	22500± 34637
10	10	8:1	300	37500	37500
10	10	8:2	300	37500	37500
10	10	8:3	<60	300	300
10	10	8:4	60	7500	7500
10	10	8:5	<60	300	300
10	10	8:6	<60	37500	37500
10	10	8:7	<60	7500	7500
10	10	8:8	<60	nt	nt
Group mean titre (mean±SD)			220± 139	18300± 18199	18300± 18199

TABLE 2

Amount ribavirin (mg/dose)	Amount immunogen (µg/dose)	Mouse ID	Antibody titre to rNS3 at indicated week		
			Week 4	Week 6	Week 8
None	50	1:1	60	7500	7500
None	50	1:2	60	7500	7500
None	50	1:3	60	7500	7500
None	50	1:4	<60	1500	300
None	50	1:5	300	37500	37500
None	50	1:6	60	7500	7500
None	50	1:7	60	37500	7500
None	50	1:8	.	.	.
Group mean titre (mean±SD)			100 ±98	15214 ±15380	10757 ±12094
1	50	2:1	60	7500	7500
1	50	2:2	300	37500	7500
1	50	2:3	60	187500	7500
1	50	2:4	60	37500	187500
1	50	2:5	60	37500	7500
1	50	2:6	60	37500	37500
1	50	2:7	300	37500	7500
1	50	2:8	300	37500	37500
Group mean titre (mean±SD)			150 ±124	52500 ±55549	37500 ±62105
3	50	3:1	60	37500	7500
3	50	3:2	300	37500	37500
3	50	3:3	300	37500	7500
3	50	3:4	60	37500	7500
3	50	3:5	300	37500	7500
3	50	3:6	60	37500	7500
3	50	3:7	-	7500	37500
3	50	3:8	1500	7500	37500
Group mean titre (mean±SD)			387 ±513	30000 ±13887	18750 ±15526
10	50	4:1	300	7500	7500
10	50	4:2	300	37500	37500
10	50	4:3	60	7500	7500
10	50	4:4	60	7500	7500
10	50	4:5	60	1500	1500
10	50	4:6	60	7500	37500
10	50	4:7	-	7500	7500
10	50	8:8	60	37500	7500
Group mean titre (mean±SD)			140 ±124	10929 ±11928	15214 ±15380

TABLE 3

Group	Week	Mean±SD	Group	Mean±SD	analysis	p-value
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 1 mg ribavirin	252 ±107	Students t-test	0.4071
	6	7500 ±12421		28071 ±16195	Students t-test	0.0156
	8	3042 ±3076		36642 ±67565	Students t-test	0.2133
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 3 mg ribavirin	180± 128	Students t-test	1.000
	6	7500 ±12421		30000± 13887	Students t-test	0.0042
	8	3042 ±3076		22500± 34637	Students t-test	0.0077
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 10mg ribavirin	220± 139	Students t-test	0.7210
	6	7500 ±12421		18300± 18199	Students t-test	0.1974
	8	3042 ±3076		18300± 18199	Students t-test	0.0493

5

TABLE4

Group	Week	Mean±SD	Group	Mean±SD	analysis	p-value
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 1 mg ribavirin	150 ±124	Students t-test	0.4326
	6	15214 ±15380		52500 ±55549	Students t-test	0.1106
	8	10757 ±12094		37500 ±62105	Students t-test	0.2847
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 3 mg ribavirin	387 ±513	Students t-test	0.2355
	6	15214 ±15380		30000 ±13887	Students t-test	0.0721
	8	10757 ±12094		18750 ±15526	Students t-test	0.2915
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 10mg ribavirin	140 ±124	Students t-test	0.5490
	6	15214 ±15380		10929 ±11928	Students t-test	0.5710
	8	10757 ±12094		15214 ±15380	Students t-test	0.5579

Significance levels: NS = not significant; * = p<0.05; ** = p<0.01; *** = p<0.001

TABLE 5

Group	Week	Mean±SD	Group	Mean±SD	analysis	p-value
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 1 mg ribavirin	252 ±107	Mann-Whitney	0.4280
	6	7500 ±12421		28071 ±16195	Mann-Whitney	0.0253
	8	3042 ±3076		36642 ±67565	Mann-Whitney	0.0245
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 3 mg ribavirin	180± 128	Mann-Whitney	0.0736
	6	7500 ±12421		30000± 13887	Mann-Whitney	0.0050
	8	3042 ±3076		22500± 34637	Mann-Whitney	0.0034
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 10mg ribavirin	220± 139	Mann-Whitney	0.8986
	6	7500 ±12421		18300± 18199	Mann-Whitney	0.4346
	8	3042 ±3076		18300± 18199	Mann-Whitney	0.2102

5

TABLE 6

Group	Week	Mean±SD	Group	Mean±SD	analysis	p-value
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 1 mg ribavirin	150 ±124	Mann-Whitney	0.1128
	6	15214 ±15380		52500 ±55549	Mann-Whitney	0.0210
	8	10757 ±12094		37500 ±62105	Mann-Whitney	0.1883
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 3 mg ribavirin	387 ±513	Mann-Whitney	0.1400
	6	15214 ±15380		30000 ±13887	Mann-Whitney	0.0679
	8	10757 ±12094		18750 ±15526	Mann-Whitney	0.2091
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 10 mg ribavirin	140 ±124	Mann-Whitney	0.4292
	6	15214 ±15380		10929 ±11928	Mann-Whitney	0.9473
	8	10757 ±12094		15214 ±15380	Mann-Whitney	0.6279

Significance levels: NS = not significant; * = p<0.05; ** = p<0.01; *** = p<0.001

The data above demonstrate that ribavirin facilitates or enhances an immune response to an HCV antigen or HCV epitopes. A potent immune response to rNS3 was elicited after immunization with a vaccine composition comprising as little as 1 mg ribavirin and 10 µg of rNS3 antigen. The data above also provide evidence that the amount of ribavirin that is sufficient to facilitate an immune response to an antigen is between 1 and 3 mg per injection for a 25-30g Balb/c mouse. It should be realized, however, that these amounts are intended for guidance only and should not be interpreted to limit the scope of the invention in any way. Nevertheless, the data shows that vaccine compositions comprising approximately 1 to 3 mg doses of ribavirin induce an immune response that is more than 12 times higher than the immune response elicited in the absence of ribavirin (TABLES 3 and 4). Thus, ribavirin has a significant adjuvant effect on the humoral immune response of an animal and thereby, enhances or facilitates the immune response to the antigen. The example below describes experiments that were performed to better understand the amount of ribavirin needed to enhance or facilitate an immune response to an antigen.

EXAMPLE 2

To determine a dose of ribavirin that is sufficient to provide an adjuvant effect, the following experiments were performed. In a first set of experiments, groups of mice (three per group) were immunized with a 20µg rNS3 alone or a mixture of 20µg rNS3 and 0.1mg, 1mg, or 10mg ribavirin. The levels of antibody to the antigen were then determined by EIA. The mean endpoint titers at weeks 1 and 3 were plotted and are shown in FIGURE 2. It was discovered that the adjuvant effect provided by ribavirin had different kinetics depending on the dose of ribavirin provided. For example, even low doses (<1mg) of ribavirin were found to enhance antibody levels at week one but not at week three, whereas, higher doses (1-10mg) were found to enhance antibody levels at week three.

A second set of experiments was also performed. In these experiments, groups of mice were injected with vaccine compositions comprising various amounts of ribavirin and rNS3 and the IgG response in these animals was monitored. The vaccine compositions comprised approximately 100 µl phosphate buffered saline and 20 µg rNS3 with or without 0.1 mg, 1.0 mg, or 10 mg ribavirin (Sigma). The mice were bled at week six and rNS3-specific IgG levels were determined by EIA as described previously. As shown in TABLE 7, the adjuvant effects on the sustained antibody levels were most obvious in the dose range of 1 to 10 mg per injection for a 25-30g mouse.

TABLE 7

Immunogen	Amount (mg) ribavirin mixed with the immunogen	Mouse ID	Endpoint titre of rNS3 IgG at indicated week		
			Week 1	Week 2	Week 3
20 µg rNS3	None	1	60	360	360
20 µg rNS3	None	2	360	360	2160
20 µg rNS3	None	3	360	2160	2160
		Mean	260±173	960±1039	1560±1039
20 µg rNS3	0.1	4	2160	12960	2160
20 µg rNS3	0.1	5	60	60	60
20 µg rNS3	0.1	6	<60	2160	2160
			1110±1484	5060±6921	1460±1212
20 µg rNS3	1.0	7	<60	60	12960
20 µg rNS3	1.0	8	<60	2160	2160
20 µg rNS3	1.0	9	360	2160	2160
		Mean	360	1460±1212	5760±6235
20 µg rNS3	10.0	10	360	12960	77760
20 µg rNS3	10.0	11	<60	2160	12960
20 µg rNS3	10.0	12	360	2160	2160
		Mean	360	5760±6235	30960±40888

- 5 In a third set of experiments, the adjuvant effect of ribavirin after primary and booster injections was investigated. In these experiments, mice were given two intraperitoneal injections of a vaccine composition comprising 10 µg rNS3 with or without ribavirin and the IgG subclass responses to the antigen was monitored, as before. Accordingly, mice were immunized with 100 µl phosphate buffered containing 10 µg recombinant NS3 alone, with or without 0.1 or 1.0 mg
- 10 ribavirin (Sigma) at weeks 0 and 4. The mice were bled at week six and NS3-specific IgG subclasses were determined by EIA as described previously. As shown in TABLE 8, the addition of ribavirin to the immunogen prior to the injection does not change the IgG subclass response in the NS3-specific immune response. Thus, the adjuvant effect of a vaccine composition comprising ribavirin and an antigen can not be explained by a shift in the Th1/Th2-balance. It appears that
- 15 another mechanism may be responsible for the adjuvant effect of ribavirin.

TABLE 8

Immunogen	Amount (mg) ribavirin mixed with the immunogen	Mouse ID	Endpoint titre of indicated NS3 IgG subclass			
			IgG1	IgG2a	IgG2b	IgG3
10 µg rNS3	None	1	360	60	<60	60
10 µg rNS3	None	2	360	<60	<60	60
10 µg rNS3	None	3	2160	60	<60	360
		Mean	960±1039	60	-	160±173
10 µg rNS3	0.1	4	360	<60	<60	60
10 µg rNS3	0.1	5	60	<60	<60	<60
10 µg rNS3	0.1	6	2160	60	60	360
			860±1136	60	60	210±212
10 µg rNS3	1.0	7	2160	<60	<60	60
10 µg rNS3	1.0	8	360	<60	<60	<60
10 µg rNS3	1.0	9	2160	<60	<60	60
		Mean	1560±1039	-	-	60

- 5 The data presented in this example further verify that ribavirin can be administered as an adjuvant and establish that that the dose of ribavirin can modulate the kinetics of the adjuvant effect. The example below describes another assay that was performed to evaluate the ability of ribavirin to enhance or facilitate an immune response to an antigen.

EXAMPLE 3

- 10 This assay can be used with any ribavirin derivative or combinations of ribavirin derivatives to determine the extent that a particular vaccine formulation modulates a cellular immune response. To determine CD4⁺ T cell responses to a ribavirin-containing vaccine, groups of mice were immunized *s.c.* with either 100µg rNS3 in PBS or 100µg rNS3 and 1mg ribavirin in PBS. The mice were sacrificed ten days post-immunization and their lymph nodes were harvested
- 15 and drained. *In vitro* recall assays were then performed. (See e.g., Hultgren et al., *J Gen Virol.* 79:2381-91 (1998) and Hultgren et al., *Clin. Diagn. Lab. Immunol.* 4:630-632 (1997)). The amount of CD4⁺ T cell proliferation was determined at 96 h of culture by the incorporation of [³H] thymidine.

- 20 As shown in FIGURE 3, mice that were immunized with 100µg rNS3 mixed with 1mg ribavirin had a much greater T cell proliferative response than mice that were immunized with 100µg rNS3 in PBS. These data provide additional evidence that ribavirin enhances or facilitates a cellular immune response (e.g., by promoting the effective priming of T cells). The section

below discusses some of the antigens and epitopes that can be used with the embodiments described herein.

Antigens and epitopes

Virtually any antigen that can be used to generate an immune response in an animal can be combined with ribavirin so as to prepare the compositions described herein. That is, antigens that
5 can be incorporated into such compositions (e.g., vaccines) comprise bacterial antigens or epitopes, fungal antigens or epitopes, plant antigens or epitopes, mold antigens or epitopes, viral antigens or epitopes, cancer cell antigens or epitopes, toxin antigens or epitopes, chemical antigens or epitopes, and self-antigens or epitopes. Although many of these molecules induce a significant immune
10 response without an adjuvant, ribavirin can be administered in conjunction with or combined with "strong" or "weak" antigens or epitopes to enhance or facilitate the immune response to said antigen or epitope. In addition, the use of ribavirin as an adjuvant may allow for the use of lesser amounts of antigens while retaining immunogenicity.

In addition to peptide antigens, nucleic acid-based antigens can be used in the vaccine
15 compositions described herein. Various nucleic acid-based vaccines are known and it is contemplated that these compositions and approaches to immunotherapy can be augmented by reformulation with ribavirin (See e.g., U.S. Pat. No. 5,589,466 and 6,235,888). By one approach, for example, a gene encoding a polypeptide antigen of interest is cloned into an expression vector capable of expressing the polypeptide when introduced into a subject. The expression construct is
20 introduced into the subject in a mixture of ribavirin or in conjunction with ribavirin (e.g., ribavirin is administered shortly after the expression construct at the same site). Alternatively, RNA encoding a polypeptide antigen of interest is provided to the subject in a mixture with ribavirin or in conjunction with ribavirin.

Where the antigen is to be DNA (e.g., preparation of a DNA vaccine composition), suitable
25 promoters include Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, Cytomegalovirus (CMV) such as the CMV immediate early promoter, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human actin, human myosin, human hemoglobin, human muscle creatine and human
30 metallothionein can be used. Examples of polyadenylation signals useful with some embodiments, especially in the production of a genetic vaccine for humans, include but are not limited to, SV40 polyadenylation signals and LTR polyadenylation signals. In particular, the SV40 polyadenylation signal, which is in pCEP4 plasmid (Invitrogen, San Diego Calif.), referred to as the SV40 polyadenylation signal, is used.

In addition to the regulatory elements required for gene expression, other elements may also be included in a gene construct. Such additional elements include enhancers. The enhancer may be selected from the group including but not limited to: human actin, human myosin, human hemoglobin, human muscle creatine and viral enhancers such as those from CMV, RSV and EBV.

5 Gene constructs can be provided with mammalian origin of replication in order to maintain the construct extrachromosomally and produce multiple copies of the construct in the cell. Plasmids pCEP4 and pREP4 from Invitrogen (San Diego, CA) contain the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which produces high copy episomal replication without integration. All forms of DNA, whether replicating or non-replicating, which

10 do not become integrated into the genome, and which are expressible, can be used. The example below describes the use of a composition comprising a nucleic acid-based antigen and ribavirin.

EXAMPLE 4

The following describes the immunization of an animal with a vaccine comprising a nucleic acid-based antigen and ribavirin. Five to six week old female and male Balb/C mice are

15 anesthetized by intraperitoneal injection with 0.3ml of 2.5% Avertin. A 1.5cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. One group of mice are injected with approximately 20:g of an expression construct having the gp-120 gene, driven by a cytomegalovirus (CMV) promotor and second group of mice are injected with approximately 5:g of capped *in vitro* transcribed RNA (e.g., SP6, T7, or T3 (Ambion)) encoding gp-120. These two

20 groups are controls. A third group of mice is injected with approximately 20:g of the expression vector having the gp-120 gene and the CMV promoter mixed with 1mg of ribavirin and a fourth group of mice is injected with approximately 5:g of capped *in vitro* transcribed RNA mixed with 1mg ribavirin. The vaccines are injected in 0.1ml of solution (PBS) in a 1 cc syringe through a 27

25 gauge needle over one minute, approximately 0.5cm from the distal insertion site of the muscle into the knee and about 0.2cm deep. A suture is placed over the injection site for future localization, and the skin is then closed with stainless steel clips.

Blood samples are obtained prior to the injection (Day 0) and up to more than 40 days post injection. The serum from each sample is serially diluted and assayed in a standard ELISA technique assay for the detection of antibody, using recombinant gp-120 protein made in yeast as

30 the antigen. Both IgG and IgM antibodies specific for gp-120 will be detected in all samples, however, groups three and four, which contained the ribavirin, will exhibit a greater immune response to the gp-120 as measured by the amount and/or titer of antibody detected in the sera.

Preferred embodiments of the invention comprise ribavirin and a viral antigen or an epitope present on a virus, preferably a hepatitis virus. Compositions comprise, for example,

35 ribavirin and an HAV antigen, HBV antigen, HCV antigen or any combination of these antigens or

epitopes present on one or more of these viruses. The hepatitis antigens can be peptides or nucleic acids. Compositions that can be used to vaccinate against HAV infection, for example, comprise ribavirin and an HAV peptide with a length of at least 3-10 consecutive amino acids, 10-50 consecutive amino acids, 50-100 consecutive amino acids, 100-200 consecutive amino acids, 200-400 consecutive amino acids, 400-800 consecutive amino acids, 800-1200 consecutive amino acids, 1200-1600 consecutive amino acids, 1600-2000 consecutive amino acids, and 2000-2227 consecutive amino acids of SEQ. ID. NO.: 12.

Additionally, compositions comprising ribavirin and a nucleic acid encoding one or more of the HAV peptides, described above, can be used to treat or prevent HAV infection. Preferred nucleic acid-based antigens include a nucleotide sequence of at least 9 consecutive nucleotides of an HAV sequence (e.g., SEQ. ID. NO.: 15). That is, a nucleic acid based antigen can comprise at least 9-25 consecutive nucleotides, 25-50 consecutive nucleotides, 50-100 consecutive nucleotides, 100-200 consecutive nucleotides, 200-500 consecutive nucleotides, 500-1000 consecutive nucleotides, 1000-2000 consecutive nucleotides, 2000-4000 consecutive nucleotides, 4000-8000 consecutive nucleotides, and 8000-9416 consecutive nucleotides of SEQ. ID. NO.: 15 or an RNA that corresponds to these sequences.

Similarly, preferred HBV vaccine embodiments comprise ribavirin and a HBV peptide of at least 3 consecutive amino acids of HBsAg (SEQ. ID. NO.: 10) or HBcAg and HBsAg (SEQ. ID. NO.: 11). That is, some embodiments have ribavirin and a HBV peptide with a length of at least 3-10 consecutive amino acids, 10-50 consecutive amino acids, 50-100 consecutive amino acids, 100-150 consecutive amino acids, 150-200 consecutive amino acids, and 200-226 consecutive amino acids of either SEQ. ID. NO.: 10 or SEQ. ID. NO.: 11.

Additionally, compositions comprising ribavirin and a nucleic acid encoding one or more of the HBV peptides, described above, can be used to treat or prevent HBV infection. Preferred nucleic acid-based antigens include a nucleotide sequence of at least 9 consecutive nucleotides of an HBV (e.g., SEQ. ID. NO.: 14). That is, a nucleic acid based antigen can comprise at least 9-25 consecutive nucleotides, 25-50 consecutive nucleotides, 50-100 consecutive nucleotides, 100-200 consecutive nucleotides, 200-500 consecutive nucleotides, 500-1000 consecutive nucleotides, 1000-2000 consecutive nucleotides, 2000-4000 consecutive nucleotides, 4000-8000 consecutive nucleotides, and 8000-9416 consecutive nucleotides of SEQ. ID. NO.: 14 or an RNA that corresponds to these sequences. The example below describes the use of ribavirin in conjunction with a commercial HBV vaccine preparation.

EXAMPLE 5

The adjuvant effect of ribavirin was tested when mixed with two doses of a commercially available vaccine containing HBsAg and alum. (Engerix, SKB). Approximately 0.2µg or 2µg of

Engerix vaccine was mixed with either PBS or 1mg ribavirin in PBS and the mixtures were injected intra peritoneally into groups of mice (three per group). A booster containing the same mixture was given on week four and all mice were bled on week six. The serum samples were diluted from 1:60 to 1:37500 and the dilutions were tested by EIA, as described above, except that purified human HBsAg was used as the solid phase antigen. As shown in TABLE 9, vaccine formulations having ribavirin enhanced the response to 2µg of an existing vaccine despite the fact that the vaccine already contained alum. That is, by adding ribavirin to a suboptimal vaccine dose (i.e., one that does not induce detectable antibodies alone) antibodies became detectable, providing evidence that the addition of ribavirin allows for the use of lower antigen amounts in a vaccine formulation without compromising the immune response.

TABLE 9

Week	Endpoint antibody titer to HBsAg in EIA											
	0.02µg Engerix						0.2µg Engerix					
	No ribavirin			1mg ribavirin			No ribavirin			1mg ribavirin		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
6	<60	<60	<60	<60	<60	<60	<60	<60	<60	300	60	<60

15

Some HCV vaccine compositions comprise ribavirin and a HCV peptide of at least 3 consecutive amino acids of SEQ. ID. NO.: 1 or a nucleic acid encoding said HCV peptide. That is, a vaccine composition can comprise ribavirin and one or more HCV peptides with a length of at least 3-10 consecutive amino acids, 10-50 consecutive amino acids, 50-100 consecutive amino acids, 100-200 consecutive amino acids, 200-400 consecutive amino acids, 400-800 consecutive amino acids, 800-1200 consecutive amino acids, 1200-1600 consecutive amino acids, 1600-2000 consecutive amino acids, 2000-2500 consecutive amino acids, and 2500-3011 consecutive amino acids of SEQ. ID. NO.: 1 or a nucleic acid encoding one or more of said fragments.

Preferred HCV compositions comprise ribavirin and a peptide of at least 3 consecutive amino acids of HCV core protein (SEQ. ID. NO.: 2), HCV E1 protein (SEQ. ID. NO.: 3), HCV E2 protein (SEQ. ID. NO.: 4), HCV NS2 (SEQ. ID. NO.: 5), HCV NS3 (SEQ. ID. NO.: 6), HCV NS4A (SEQ. ID. NO.: 7), HCV NS4B (SEQ. ID. NO.: 8), or HCV NS5A/B (SEQ. ID. NO.: 9) or peptides consisting of combinations of these domains. That is, preferred HCV vaccines comprise

ribavirin and a peptide with a length of at least 3-10 consecutive amino acids, 10-50 consecutive amino acids, 50-100 consecutive amino acids, 100-200 consecutive amino acids, 200-400 consecutive amino acids, 400-800 consecutive amino acids, and 800-1040 consecutive amino acids of any one or more of (SEQ. ID. NOs.: 2-9). These domains correspond to amino acid residues 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 of SEQ. ID. NO.: 1. Thus, preferred embodiments also include one or more of 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 of SEQ. ID. NO.: 1 or fragments thereof.

Vaccine compositions comprising ribavirin and a nucleic acid encoding one or more of the peptides described above are also embodiments. Preferred nucleic acid-based antigens include a nucleotide sequence of at least 9 consecutive nucleotides of HCV (SEQ. ID. NO.: 13). That is, a nucleic acid based antigen can comprise at least 9-25 consecutive nucleotides, 25-50 consecutive nucleotides, 50-100 consecutive nucleotides, 100-200 consecutive nucleotides, 200-500 consecutive nucleotides, 500-1000 consecutive nucleotides, 1000-2000 consecutive nucleotides, 2000-4000 consecutive nucleotides, 4000-8000 consecutive nucleotides, and 8000-9416 consecutive nucleotides of any one of SEQ. ID. NOs.: 13 or an RNA that corresponds to these sequences. The section below discusses some of the compositions containing ribavirin and an antigen.

Compositions containing ribavirin and an antigen

Compositions (e.g., vaccines) that comprise ribavirin and an antigen or epitope of a pathogen (e.g., virus, bacteria, mold, yeast, and parasite) may contain other ingredients including, but not limited to, adjuvants, binding agents, excipients such as stabilizers (to promote long term storage), emulsifiers, thickening agents, salts, preservatives, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. These compositions are suitable for treatment of animals either as a preventive measure to avoid a disease or condition or as a therapeutic to treat animals already afflicted with a disease or condition.

Many other ingredients can be present in the vaccine. For example, the ribavirin and antigen can be employed in admixture with conventional excipients (e.g., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral (e.g., oral) or topical application that do not deleteriously react with the ribavirin and/or antigen). Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatine, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc. Many more suitable carriers are described in *Remington's Pharmaceutical Sciences*, 15th Edition, Easton: Mack Publishing Company, pages

1405-1412 and 1461-1487(1975) and The National *Formulary* XIV, 14th Edition, Washington, American Pharmaceutical Association (1975).

The gene constructs described herein may be formulated with or administered in conjunction with agents that increase uptake and/or expression of the gene construct by the cells relative to uptake and/or expression of the gene construct by the cells that occurs when the identical genetic vaccine is administered in the absence of such agents. Such agents and the protocols for administering them in conjunction with gene constructs are described in U.S. Ser. No. 08/008,342 filed Jan. 26, 1993, U.S. Ser. No. 08/029,336 filed Mar. 11, 1993, U.S. Ser. No. 08/125,012 filed Sep. 21, 1993, PCT Patent Application Serial Number PCT/US94/00899 filed Jan. 26, 1994, and U.S. Ser. No. 08/221,579 filed Apr. 1, 1994. Examples of such agents include: CaPO₄, DEAE dextran, anionic lipids; extracellular matrix-active enzymes; saponins; lectins; estrogenic compounds and steroidal hormones; hydroxylated lower alkyls; dimethyl sulfoxide (DMSO); urea; and benzoic acid esters anilides, amidines, urethanes and the hydrochloride salts thereof such as those of the family of local anesthetics. In addition, the gene constructs are encapsulated within/administered in conjunction with lipids/polycationic complexes.

Vaccines can be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like that do not deleteriously react with ribavirin or the antigen.

The effective dose and method of administration of a particular vaccine formulation can vary based on the individual patient and the type and stage of the disease, as well as other factors known to those of skill in the art. Therapeutic efficacy and toxicity of the vaccines can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED₅₀ (the dose therapeutically effective in 50% of the population). The data obtained from cell culture assays and animal studies can be used to formulate a range of dosage for human use. The dosage of the vaccines lies preferably within a range of circulating concentrations that include the ED₅₀ with no toxicity. The dosage varies within this range depending upon the type of ribavirin derivative and antigen, the dosage form employed, the sensitivity of the patient, and the route of administration.

Since ribavirin has been on the market for several years, many dosage forms and routes of administration are known. All known dosage forms and routes of administration can be provided within the context of the embodiments described herein. Preferably, an amount of ribavirin that is effective to enhance an immune response to an antigen in an animal can be considered to be an amount that is sufficient to achieve a blood serum level of antigen approximately 0.25 - 12.5 µg/ml in the animal, preferably, about 2.5 µg/ml. In some embodiments, the amount of ribavirin is

determined according to the body weight of the animal to be given the vaccine. Accordingly, the amount of ribavirin in a vaccine formulation can be from about 0.1 - 6.0mg/kg body weight. That is, some embodiments have an amount of ribavirin that corresponds to approximately 0.1 - 1.0mg/kg, 1.1 - 2.0mg/kg, 2.1 - 3.0mg/kg, 3.1 - 4.0mg/kg, 4.1 - 5.0mg/kg, 5.1, and 6.0mg/kg body weight of an animal. More conventionally, the vaccines contain approximately 0.25mg - 2000mg of ribavirin. That is, some embodiments have approximately 250µg, 500µg, 1mg, 25mg, 50mg, 100mg, 150mg, 200mg, 250mg, 300mg, 350mg, 400mg, 450mg, 500mg, 550mg, 600mg, 650mg, 700mg, 750mg, 800mg, 850mg, 900mg, 1g, 1.1g, 1.2g, 1.3g, 1.4g, 1.5g, 1.6g, 1.7g, 1.8g, 1.9g, and 2g of ribavirin.

Conventional vaccine preparations can be modified by adding an amount of ribavirin that is sufficient to enhance an immune response to the antigen. That is, existing conventional vaccine formulations can be modified by simply adding ribavirin to the preparation or by administering the conventional vaccine in conjunction with ribavirin (e.g., shortly before or after providing the antigen). As one of skill in the art will appreciate, the amount of antigens in a vaccine can vary depending on the type of antigen and its immunogenicity. The amount of antigens in the vaccines can vary accordingly. Nevertheless, as a general guide, the vaccines can have approximately 0.25mg - 5mg, 5-10mg, 10-100mg, 100-500mg, and upwards of 2000mg of an antigen (e.g., a hepatitis viral antigen).

In some approaches described herein, the exact amount of ribavirin and/or antigen is chosen by the individual physician in view of the patient to be treated. Further, the amounts of ribavirin can be added in combination with or separately from the same or equivalent amount of antigen and these amounts can be adjusted during a particular vaccination protocol so as to provide sufficient levels in light of patient-specific or antigen-specific considerations. In this vein, patient-specific and antigen-specific factors that can be taken into account include, but are not limited to, the severity of the disease state of the patient, age, and weight of the patient, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. The next section describes the discovery of a novel HCV gene and the creation of mutant HCV sequences, which can be used with the embodiments described herein.

Novel NS3/4A and mutant NS3/4A sequences

A novel nucleic acid and protein corresponding to the NS3/4A domain of HCV was cloned from a patient infected with HCV (SEQ. ID. NOs.: 16 and 17). A Genebank search revealed that the cloned sequence had the greatest homology to HCV sequences but was only 93% homologous to the closest HCV relative (accession no AJ 278830). A truncated mutant of the novel NS3/4A peptide and NS3/4A mutants, which lack a proteolytic cleavage site, were also created. It was discovered that these novel peptides and nucleic acids encoding said peptides were potent

immunogens that can be mixed with fibavirin so as to make a composition that provides a recipient with a potent immune response to HCV. The cloning of the novel NS3/4A domain and the creation of the various NS3/4A mutants is described in the following example.

EXAMPLE 6

5 The NS3/4A sequence was amplified from the serum of an HCV-infected patient (HCV genotype 1a) using the Polymerase Chain Reaction (PCR). Total RNA was extracted from serum, cDNA synthesis, and PCR was performed according to standard protocols (Chen M et al., *J. Med. Virol.* 43:223-226 (1995)). The cDNA synthesis was initiated using the antisense primer "NS4KR" (5'-CCG TCT AGA TCA GCA CTC TTC CAT TTC ATC-3' (SEQ. ID. NO.: 18)). From this
10 cDNA, a 2079 base pair DNA fragment of HCV, corresponding to amino acids 1007 to 1711, which encompasses the NS3 and NS4A genes, was amplified. A high fidelity polymerase (Expand High Fidelity PCR, Boehringer-Mannheim, Mannheim, Germany) was used with the "NS3KF" primer (5'-CCT GAA TTC ATG GCG CCT ATC ACG GCC TAT-3' (SEQ. ID. NO.: 19) and the NS4KR primer. The NS3KF primer contained a *EcoRI* restriction enzyme cleavage site and a start
15 codon and the primer NS4KR contained a *XbaI* restriction enzyme cleavage site and a stop codon.

The amplified fragment was then sequenced SEQ. ID. NO.: 16. Sequence comparison analysis revealed that the gene fragment was indeed amplified from a viral strain of genotype 1a. A computerized BLAST search against the Genbank database using the NCBI website revealed that the closest HCV homologue was 93% identical in nucleotide sequence.

20 The amplified DNA fragment was then digested with *EcoRI* and *XbaI*, and was inserted into a pcDNA3.1/His plasmid (Invitrogen) digested with the same enzymes. The NS3/4A-pcDNA3.1 plasmid was then digested with *EcoRI* and *XbaI* and the insert was purified using the QiaQuick kit (Qiagen, Hamburg, Germany) and was ligated to a *EcoRI/XbaI* digested pVAX vector (Invitrogen) so as to generate the NS3/4A-pVAX plasmid.

25 The rNS3 truncated mutant was obtained by deleting NS4A sequence from the NS3/4A DNA. Accordingly, the NS3 gene sequence of NS3/4A-pVAX was PCR amplified using the primers NS3KF and 3'*NotI* (5'-CCA CGC GGC CGC GAC GAC CTA CAG-3' (SEQ. ID. NO.: 20)) containing *EcoRI* and *NotI* restriction sites, respectively. The NS3 fragment (1850 bp) was then ligated to a *EcoRI* and *NotI* digested pVAX plasmid to generate the NS3-pVAX vector.
30 Plasmids were grown in BL21 *E.coli* cells. The plasmids were sequenced and were verified by restriction cleavage and the results were as to be expected based on the original sequence.

To change the proteolytic cleavage site between NS3 and NS4A, the NS3/4A-pVAX plasmid was mutagenized using the QUICKCHANGE™ mutagenesis kit (Stratagene), following the manufacturer's recommendations. To generate the "TPT" mutation, the plasmid was amplified
35 using the primers 5'-CTGGAGGTCGTCACGCCTACCTGGGTGCTCGTT-3' (SEQ. ID. NO.:

21) and 5'-ACCGAGCACCCAGGTAGGCGTGACGACCTCCAG-3' (SEQ. ID. NO.: 22) resulting in NS3/4A-TPT-pVAX. To generate the "RGT" mutation, the plasmid was amplified using the primers 5'-CTGGAGGTCGTCCGCGGTACCTGGGTGCTCGTT-3' (SEQ. ID. NO.: 23) and 5'-ACCGAGCACCCAGGTACC-GCGGACGACCTCCAG-3' (SEQ. ID. NO.: 24) resulting in NS3/4A-RGT-pVAX.

All mutagenized constructs were sequenced to verify that the mutations had been correctly made. Plasmids were grown in competent BL21 E. coli. The plasmid DNA used for *in vivo* injection was purified using Qiagen DNA purification columns, according to the manufacturers instructions (Qiagen GmbH, Hilden, FRG). The concentration of the resulting plasmid DNA was determined spectrophotometrically (Dynaquant, Pharmacia Biotech, Uppsala, Sweden) and the purified DNA was dissolved in sterile phosphate buffer saline (PBS) at concentrations of 1 mg/ml. The amino acid sequences of the wild-type and mutated junctions are shown in TABLE 10. The section below describes several nucleic acids that encode HCV peptides.

TABLE 10

Plasmid	Deduced amino acid sequence
*NS3/4A-pVAX	TKYMTCMSADLEVVTSTWVLVGGVL (SEQ. ID. NO.: 25)
NS3/4A-TGT-pVAX	TKYMTCMSADLEVVTGTWVLVGGVL (SEQ. ID. NO.: 26)
NS3/4A-RGT-pVAX	TKYMTCMSADLEVVRGTWVLVGGVL (SEQ. ID. NO.: 27)
NS3/4A-TPT-pVAX	TKYMTCMSADLEVVTPTWVLVGGVL (SEQ. ID. NO.: 33)
NS3/4A-RPT-pVAX	TKYMTCMSADLEVVRPTWVLVGGVL (SEQ. ID. NO.: 34)
NS3/4A-RPA-pVAX	TKYMTCMSADLEVVRPAWVLVGGVL (SEQ. ID. NO.: 35)
NS3/4A-CST-pVAX	TKYMTCMSADLEVVCSTWVLVGGVL (SEQ. ID. NO.: 36)
NS3/4A-CCST-pVAX	TKYMTCMSADLEVCCSTWVLVGGVL (SEQ. ID. NO.: 37)
NS3/4A-SSST-pVAX	TKYMTCMSADLEVSSSTWVLVGGVL (SEQ. ID. NO.: 38)
NS3/4A-SSSSCST-pVAX	TKYMTCMSADSSSSCSTWVLVGGVL (SEQ. ID. NO.: 39)
NS3A/4A-VVVVTST-pVAX	TKYMTCMSADVVTSTWVLVGGVL (SEQ. ID. NO.: 40)
NS5-pVAX	ASEDVVCCSMSTWTG (SEQ. ID. NO.: 41)
NS5A/B-pVAX	SSEDVVCCSMWVLVGGVL (SEQ. ID. NO.: 42)

*The wild type sequence for the NS3/4A fragment is NS3/4A-pVAX. The NS3/4A breakpoint is identified by underline, wherein the P1 position corresponds to the first Thr (T) and the P1' position corresponds to the next following amino acid the NS3/4A-pVAX sequence. In the wild type NS3/4A sequence the NS3 protease cleaves between the P1 and P1' positions.

Nucleic acids encoding HCV peptides

The nucleic acid embodiments include nucleotides encoding the HCV peptides described herein (e.g., SEQ. ID. NO.: 17, 29, 31, 32, and 43-49) or fragments thereof at least 4, 6, 8, 10, 12, 15, or 20 amino acids in length (e.g., SEQ. ID. NOs.: 25-27, and 33-42). Some embodiments for

example, include genomic DNA, RNA, and cDNA encoding these HCV peptides. The HCV nucleotide embodiments not only include the DNA sequences shown in the sequence listing (e.g., SEQ. ID. NO.: 16) but also include nucleotide sequences encoding the amino acid sequences shown in the sequence listing (e.g., SEQ. ID. NO.: 17) and any nucleotide sequence that hybridizes to the DNA sequences shown in the sequence listing under stringent conditions (e.g., hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7.0% sodium dodecyl sulfate (SDS), 1 mM EDTA at 50°C) and washing in 0.2 X SSC/0.2% SDS at 50°C and any nucleotide sequence that hybridizes to the DNA sequences that encode an amino acid sequence provided in the sequence listing (SEQ. ID. NOS.: 17) under less stringent conditions (e.g., hybridization in 0.5 M NaHPO₄, 7.0% sodium dodecyl sulfate (SDS), 1 mM EDTA at 37°C and washing in 0.2X SSC/0.2% SDS at 37°C).

The nucleic acid embodiments also include fragments, modifications, derivatives, and variants of the sequences described above. Desired embodiments, for example, include nucleic acids having at least 12 consecutive bases of one of the novel HCV sequences or a sequence complementary thereto and preferred fragments include at least 12 consecutive bases of a nucleic acid encoding the NS3/4A molecule of SEQ. ID. NO.: 17 or a sequence complementary thereto.

In this regard, the nucleic acid embodiments of the invention can have from 12 to approximately 2079 consecutive nucleotides. Some DNA fragments of the invention, for example, include nucleic acids having at least 12-15, 15-20, 20-30, 30-50, 50-100, 100-200, 200-500, 500-1000, 1000-1500, 1500-2079 consecutive nucleotides of SEQ. ID. NO.: 16 or a complement thereof. The nucleic acid embodiments can also be altered by mutation such as substitutions, additions, or deletions. Due to the degeneracy of nucleotide coding sequences, for example, other DNA sequences that encode substantially the same HCV amino acid sequence as depicted in SEQ. ID. NOS: 17 can be used in some embodiments. These include, but are not limited to, nucleic acid sequences encoding all or portions of NS3/4A (SEQ. ID. NO.: 16) or nucleic acids that complement all or part of this sequence that have been altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change, or a functionally non-equivalent amino acid residue within the sequence, thus producing a detectable change.

By using the nucleic acid sequences described above, probes that complement these molecules can be designed and manufactured by oligonucleotide synthesis. Desirable probes comprise a nucleic acid sequence of (SEQ. ID. NO.: 16) that is unique to this HCV isolate. These probes can be used to screen cDNA from patients so as to isolate natural sources of HCV, some of which may be novel HCV sequences in themselves. Screening can be by filter hybridization or by PCR, for example. By filter hybridization, the labeled probe preferably contains at least 15-30

base pairs of the nucleic acid sequence of (SEQ. ID. NO.: 16) that is unique to to this NS3/4A peptide. The hybridization washing conditions used are preferably of a medium to high stringency. The hybridization can be performed in 0.5M NaHPO₄, 7.0% sodium dodecyl sulfate (SDS), 1 mM EDTA at 42°C overnight and washing can be performed in 0.2X SSC/0.2% SDS at 42°C. For
5 guidance regarding such conditions see, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y.

HCV nucleic acids can also be isolated from patients infected with HCV using the nucleic acids described herein. (See also *Example 6*). Accordingly, RNA obtained from a patient infected
10 with HCV is reverse transcribed and the resultant cDNA is amplified using PCR or another amplification technique. The primers are preferably obtained from the NS3/4A sequence (SEQ. ID. NO.: 16).

For a review of PCR technology, see *Molecular Cloning to Genetic Engineering*, White, B.A. Ed. in Methods in Molecular Biology 67: Humana Press, Totowa (1997) and the publication entitled
15 "PCR Methods and Applications" (1991, Cold Spring Harbor Laboratory Press). For amplification of mRNAs, it is within the scope of the invention to reverse transcribe mRNA into cDNA followed by PCR (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770. Another technique involves the use of Reverse Transcriptase Asymmetric Gap Ligase Chain Reaction (RT-AGLCR), as described by Marshall R.L. et al. (*PCR Methods and*
20 *Applications* 4:80-84, 1994).

Briefly, RNA is isolated, following standard procedures. A reverse transcription reaction is performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment as a primer of first strand synthesis. The resulting RNA/DNA hybrid is then "tailed" with guanines using a standard terminal transferase reaction. The hybrid is then digested
25 with RNase H, and second strand synthesis is primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment are easily isolated. For a review of cloning strategies which can be used, see e.g., Sambrook et al., 1989, *supra*.

In each of these amplification procedures, primers on either side of the sequence to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable
30 polymerase, such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are then extended. Thereafter, another cycle of denaturation, hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites. PCR

has further been described in several patents including US Patents 4,683,195, 4,683,202 and 4,965,188.

The primers are selected to be substantially complementary to a portion of the nucleic acid sequence of (SEQ. ID. NO.: 16) that is unique to this NS3/4A molecule, thereby allowing the sequences between the primers to be amplified. Preferably, primers are at least 16-20, 20-25, or 25-30 nucleotides in length. The formation of stable hybrids depends on the melting temperature (T_m) of the DNA. The T_m depends on the length of the primer, the ionic strength of the solution and the G+C content. The higher the G+C content of the primer, the higher is the melting temperature because G:C pairs are held by three H bonds whereas A:T pairs have only two. The G+C content of the amplification primers described herein preferably range between 10 and 75 %, more preferably between 35 and 60 %, and most preferably between 40 and 55 %. The appropriate length for primers under a particular set of assay conditions can be empirically determined by one of skill in the art.

The spacing of the primers relates to the length of the segment to be amplified. In the context of the embodiments described herein, amplified segments carrying nucleic acid sequence encoding HCV peptides can range in size from at least about 25 bp to the entire length of the HCV genome. Amplification fragments from 25-1000 bp are typical, fragments from 50-1000 bp are preferred and fragments from 100-600 bp are highly preferred. It will be appreciated that amplification primers can be of any sequence that allows for specific amplification of the NS3/4A region and can, for example, include modifications such as restriction sites to facilitate cloning.

The PCR product can be subcloned and sequenced to ensure that the amplified sequences represent the sequences of an HCV peptide. The PCR fragment can then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment can be labeled and used to screen a cDNA library, such as a bacteriophage cDNA library. Alternatively, the labeled fragment can be used to isolate genomic clones via the screening of a genomic library. Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from an infected patient. In this manner, HCV gene products can be isolated using standard antibody screening techniques in conjunction with antibodies raised against the HCV gene product. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor.)

Embodiments also include (a) DNA vectors that contain any of the foregoing nucleic acid sequences and/or their complements (i.e., antisense); (b) DNA expression vectors that contain any of the foregoing nucleic acid sequences operatively associated with a regulatory element that directs the expression of the nucleic acid; and (c) genetically engineered host cells that contain any of the foregoing nucleic acid sequences operatively associated with a regulatory element that

directs the expression of the coding sequences in the host cell. These recombinant constructs are capable of replicating autonomously in a host cell. Alternatively, the recombinant constructs can become integrated into the chromosomal DNA of a host cell. Such recombinant polynucleotides typically comprise an HCV genomic or cDNA polynucleotide of semi-synthetic or synthetic origin
5 by virtue of human manipulation. Therefore, recombinant nucleic acids comprising these sequences and complements thereof that are not naturally occurring are provided.

Although nucleic acids encoding an HCV peptide or nucleic acids having sequences that complement an HCV gene as they appear in nature can be employed, they will often be altered, e.g., by deletion, substitution, or insertion and can be accompanied by sequence not present in
10 humans. As used herein, regulatory elements include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression. Such regulatory elements include, but are not limited to, the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter
15 regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α -mating factors.

In addition, recombinant HCV peptide-encoding nucleic acid sequences and their complementary sequences can be engineered so as to modify their processing or expression. For example, and not by way of limitation, the HCV nucleic acids described herein can be combined
20 with a promoter sequence and/or ribosome binding site, or a signal sequence can be inserted upstream of HCV peptide-encoding sequences so as to permit secretion of the peptide and thereby facilitate harvesting or bioavailability. Additionally, a given HCV nucleic acid can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction sites or destroy preexisting ones, or
25 to facilitate further *in vitro* modification. (See Example 6). Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis. (Hutchinson et al., *J. Biol. Chem.*, 253:6551 (1978)).

Further, nucleic acids encoding other proteins or domains of other proteins can be joined to nucleic acids encoding an HCV peptide so as to create a fusion protein. Nucleotides encoding
30 fusion proteins can include, but are not limited to, a full length NS3/4A sequence (SEQ. ID. NO.: 16), a truncated NS3/4A sequence or a peptide fragment of an NS3/4A sequence fused to an unrelated protein or peptide, such as for example, poly histidine, hemagglutinin, an enzyme, fluorescent protein, or luminescent protein, as discussed below.

Surprisingly, it was discovered that the NS3-pVAX and NS3/4A-pVAX vectors were capable of eliciting a potent immune response when injected into an immunocompetent mammal. The example below describes these experiments in greater detail.

EXAMPLE 7

5 To determine whether a humoral immune response was elicited by the NS3-pVAX and NS3/4A-pVAX vectors, the expression constructs described in *Example 6* were purified using the Qiagen DNA purification system, according to the manufacturer's instructions and the purified DNA vectors were used to immunize groups of four to ten Balb/c mice. The plasmids were injected directly into regenerating tibialis anterior (TA) muscles as previously described (Davis et al.,
10 *Human Gene Therapy* 4(6):733 (1993)). In brief, mice were injected intramuscularly with 50 μ l/TA of 0.01mM cardiotoxin (Latoxan, Rosans, France) in 0.9% sterile NaCl. Five days later, each TA muscle was injected with 50 μ l PBS containing either rNS3 or DNA.

Inbred mouse strains C57/BL6 (H-2b) Balb/C (H-2d), and CBA (H-2k) were obtained from the breeding facility at Møllegaard Denmark, Charles River Uppsala, Sweden, or B&K Sollentuna
15 Sweden. All mice were female and were used at 4-8 weeks of age. For monitoring of humoral responses, all mice received a booster injection of 50 μ l /TA of plasmid DNA every fourth week. In addition, some mice were given recombinant NS3 (rNS3) protein, which was purified as described herein. The mice receiving rNS3 were immunized no more than twice. All mice were bled twice a month.

20 Enzyme immunosorbent assays (EIAs) were used to detect the presence of murine NS3 antibodies. These assays were performed essentially as described in (Chen et al., *Hepatology* 28(1): 219 (1998)). Briefly, rNS3 was passively adsorbed overnight at 4°C to 96-well microtiter plates (Nunc, Copenhagen, Denmark) at 1 μ g/ml in 50 mM sodium carbonate buffer (pH 9.6). The plates were then blocked by incubation with dilution buffer containing PBS, 2% goat serum, and
25 1% bovine serum albumin for one hour at 37°C. Serial dilutions of mouse sera starting at 1:60 were then incubated on the plates for one hour. Bound murine serum antibodies were detected by an alkaline phosphatase conjugated goat anti-mouse IgG (Sigma Cell Products, Saint Louis, MO) followed by addition of the substrate pNPP (1 tablet/5ml of 1M Diethanol amine buffer with 0.5 mM MgCl₂). The reaction was stopped by addition of 1M NaOH and absorbency was read at 405
30 nm.

After four weeks, four out of five mice immunized with NS3/4A-pVAX had developed NS3 antibodies, whereas one out of five immunized with NS3-pVAX had developed antibodies (FIGURE 4). After six weeks, four out of five mice immunized with NS3/4A-pVAX had developed high levels ($>10^4$) of NS3 antibodies (mean levels 10800 ± 4830) and one had a titer of
35 2160. Although all mice immunized with NS3-pVAX developed NS3 antibodies, none of them

developed levels as high as that produced by the NS3/4A-pVAX construct (mean levels 1800 ± 805). The antibody levels elicited by the NS3/4A fusion construct were significantly higher than those induced by NS3-pVAX at six weeks (mean ranks 7.6 v.s 3.4, $p < 0.05$, Mann-Whitney rank sum test, and $p < 0.01$, Students t-test). Thus, immunization with either NS3-pVAX or NS3/4A-pVAX resulted in the production of anti-NS3 antibodies, but the NS3/4A fusion gene was a more potent immunogen. The example below describes experiments that were performed to determine if the NS3/4A-TPT-pVAX construct could elicit a potent immune response.

EXAMPLE 8

To test if the enhanced immunogenicity of NS3/4A could be solely attributed to the presence of NS4A, or if the NS3/4A fusion protein in addition had to be cleaved at the NS3/4A junction, new experiments were performed. In a first experiment, the immunogenicity of the NS3-pVAX, NS3/4A-pVAX, and NS3/4A-TPT-pVAX vectors were compared in Balb/c mice. Mice were immunised on week 0 as described above, and, after two weeks, all mice were bled and the presence of antibodies to NS3 at a serum dilution of 1:60 was determined (TABLE 11). Mice were bled again on week 4. Although, the NS3/4A-TPT-pVAX vector was comparable to the NS3-pVAX vector (4/10 vs. 0/10; NS, Fisher's exact test), the NS3/4A-pVAX vector continued to be the most potent immunogen. Thus, all of the HCV constructs that were introduced into mice were capable of eliciting an immune response against NS3, however, the NS4A sequence and a functional proteolytic cleavage site between the NS3 and NS4A sequences provided for a more potent immune response.

TABLE 11

Weeks from 1st immunization	No. of antibody responders to the respective immunogen after one 100 μ g <i>i.m</i> immunization		
	NS3-pVAX	NS3/4A-pVAX	NS3/4A-TPT-pVAX
2	0/10	17/20	4/10
4	0/10 (< 60)	20/20 (2415 ± 3715) 55% $> 10^3$ 10% $> 10^4$	10/10 (390 ± 639) 50% $> 10^2$ 10% $> 10^3$

During the chronic phase of infection, HCV replicates in hepatocytes, and spreads within the liver. A major factor in combating chronic and persistent viral infections is the cell-mediated immune defense system. CD4+ and CD8+ lymphocytes infiltrate the liver during the chronic

phase of HCV infection, but they are incapable of clearing the virus or preventing liver damage. In addition, persistent HCV infection is associated with the onset of hepatocellular carcinoma (HCC). The examples below describe experiments that were performed to determine whether the NS3 and NS3/4A construct were capable of eliciting a T-cell mediated immune response against NS3.

5

EXAMPLE 9

To study whether the constructs described above were capable of eliciting a cell-mediated response against NS3, an *in vivo* tumor growth assay was performed. To this end, an SP2/0 tumor cell line stably transfected with the NS3/4A gene was made. The pcDNA3.1 plasmid containing the NS3/4A gene was linearized by BglII digestion. A total of 5µg linearized plasmid DNA was mixed with 60µg transfection reagent (Superfect, Qiagen, Germany) and the mixture was added to a 50% confluent layer of SP2/0 cells in a 35 mm dish. The transfected SP2/0 cells (NS3/4A-SP2/0) were grown for 14 days in the presence of 800µg/ml geneticin and individual clones were isolated. A stable NS3/4A-expressing SP2/0 clone was identified using PCR and RTPCR. The cloned cell line was maintained in DMEM containing 10% fetal bovine serum, L-glutamine, and penicillin-streptomycin.

The *in vivo* growth kinetics of the SP2/0 and the NS3/4A-SP2/0 cell lines were then evaluated in Balb/c mice. Mice were injected subcutaneously with 2×10^6 tumor cells in the right flank. Each day the size of the tumor was determined through the skin. The growth kinetics of the two cell lines was comparable. For example, the mean tumor sizes did not differ between the two cell lines at any time point. (See TABLE 12). The example below describes experiments that were performed to determine whether mice immunized with the NS3/4A constructs had developed a T-cell response against NS3.

25

TABLE 12

Mouse ID	Tumor cell line	Maximum <i>in vivo</i> tumor size at indicated time point								
		5	6	7	8	11	12	13	14	15
1	SP2/0	1.6	2.5	4.5	6.0	10.0	10.5	11.0	12.0	12.0
2	SP2/0	1.0	1.0	2.0	3.0	7.5	7.5	8.0	11.5	11.5
3	SP2/0	2.0	5.0	7.5	8.0	11.0	11.5	12.0	12.0	13.0
4	SP2/0	4.0	7.0	8.0	10.0	13.0	15.0	16.5	16.5	17.0
5	SP2/0	1.0	1.0	3.0	4.0	5.0	6.0	6.0	6.0	7.0

Mouse ID	Tumor cell line	Maximum <i>in vivo</i> tumor size at indicated time point								
		5	6	7	8	11	12	13	14	15
Group mean		1,92	3.3	5.0	6.2	9.3	10.1	10.7	11.6	12.1
6	NS3/4A-SP2/0	1.0	2.0	3.0	3.5	4.0	5.5	6.0	7.0	8.0
7	NS3/4A-SP2/0	2.0	2.5	3.0	5.0	7.0	9.0	9.5	9.5	11.0
8	NS3/4A-SP2/0	1.0	2.0	3.5	3.5	9.5	11.0	12.0	14.0	14.0
9	NS3/4A-SP2/0	1.0	1.0	2.0	6.0	11.5	13.0	14.5	16.0	18.0
10	NS3/4A-SP2/0	3.5	6.0	7.0	10.5	15.0	15.0	15.0	15.5	20.0
Group mean		1,7	2,7	3.7	5.7	9.4	10.7	11.4	12.4	14.2
p-value of student's t-test comparison between group means		0,7736	0.6918	0.4027	0.7903	0.9670	0.7986	0.7927	0.7508	0.4623

EXAMPLE 10

To examine whether a T-cell response is elicited by the NS3/4A immunization, the capacity of an immunized mouse's immune defense system to attack the NS3-expressing tumor cell line was assayed. The protocol for testing for *in vivo* inhibition of tumor growth of the SP2/0 myeloma cell line in Balb/c mice has been described in detail previously (Encke et al., *J. Immunol.* 161:4917 (1998)). Inhibition of tumor growth in this model is dependent on the priming of cytotoxic T lymphocytes (CTLs). Briefly, groups of ten mice were immunized *i.m.* five times with one month intervals with either 100µg NS3-pVAX or 100 µg NS3/4A-pVAX. Two weeks after the last immunization 2×10^6 SP2/0 or NS3/4A-SP2/0 cells were injected into the right flank of each mouse. Two weeks later the mice were sacrificed and the maximum tumor sizes were measured. There was no difference between the mean SP2/0 and NS3/4A-SP2/0 tumor sizes in the NS3-pVAX immunized mice (See TABLE 13).

TABLE 13

Mouse ID	Immunogen	Dose (µg)	Tumor cell line	Tumor growth	Maximum tumor size (mm)
1	NS3-pVAX	100	SP2/0	Yes	5
2	NS3-pVAX	100	SP2/0	Yes	15
3	NS3-pVAX	100	SP2/0	No	-
4	NS3-pVAX	100	SP2/0	Yes	6
5	NS3-pVAX	100	SP2/0	Yes	13
Group total				4/5	9.75±4.992
6	NS3-pVAX	100	NS3/4A-SP2/0	Yes	9
7	NS3-pVAX	100	NS3/4A-SP2/0	Yes	8
8	NS3-pVAX	100	NS3/4A-SP2/0	Yes	7
9	NS3-pVAX	100	NS3/4A-SP2/0	No	-
10	NS3-pVAX	100	NS3/4A-SP2/0	No	-
				3/5	8.00±1.00

Note: Statistical analysis (StatView): Student's t-test on maximum tumor size. P-values < 0.05 are considered significant.

5

Unpaired t-test for Max diam

Grouping Variable: Column 1

Hypothesized Difference = 0

10

Row exclusion: NS3DNA-Tumor-001213

	Mean Diff.	DF	t-Value	P-Value
NS3-sp2, NS3-spNS3	1.750	5	0.58	0.584

15

Group Info for Max diam

Grouping Variable: Column 1

Row exclusion: NS3DNA-Tumor-001213

	Count	Mean	Variance	Std. Dev.	Std. Err
NS3-sp2	4	9.750	24.917	4.992	2.496
NS3-spNS3	3	8.000	1.000	1.000	0.57

20

In the next set of experiments, the inhibition of SP2/0 or NS3/4A-SP2/0 tumor growth was evaluated in NS3/4A-pVAX immunized Balb/c mice. In mice immunized with the NS3/4A-pVAX plasmid the growth of NS3/4A-SP2/0 tumor cells was significantly inhibited as compared to growth of the non-transfected SP2/0 cells. (See TABLE 14). Thus, NS3/4A-pVAX immunization elicits CTLs that inhibit growth of cells expressing NS3/4A *in vivo*. The example below describes experiments that were performed to analyze the efficiency of various NS3 containing compositions in eliciting a cell-mediated response to NS3.

25

TABLE 14

Mouse ID	Immunogen	Dose (µg)	Tumor cell line	Tumor growth	Maximum tumor size (mm)
11	NS3/4A-pVAX	100	SP2/0	No	-
12	NS3/4A-pVAX	100	SP2/0	Yes	24
13	NS3/4A-pVAX	100	SP2/0	Yes	9
14	NS3/4A-pVAX	100	SP2/0	Yes	11
15	NS3/4A-pVAX	100	SP2/0	Yes	25
				4/5	17.25±8.421
16	NS3/4A-pVAX	100	NS3/4A-SP2/0	No	-
17	NS3/4A-pVAX	100	NS3/4A-SP2/0	Yes	9
18	NS3/4A-pVAX	100	NS3/4A-SP2/0	Yes	7
19	NS3/4A-pVAX	100	NS3/4A-SP2/0	Yes	5
20	NS3/4A-pVAX	100	NS3/4A-SP2/0	Yes	4
				4/5	6.25±2.217

Note: Statistical analysis (StatView): Student's t-test on maximum tumor size. P-values < 0.05 are considered significant.

5

Unpaired t-test for Max diam

Grouping Variable: Column 1

Hypothesized Difference = 0

10

Row exclusion: NS3DNA-Tumor-001213

	Mean Diff.	DF	t-Value	P-Value
NS3/4-sp2, NS3/4-spNS3	11.000	6	2.526	0.044

15

Group Info for Max diam

Grouping Variable: Column 1

Row exclusion: NS3DNA-Tumor-001213

	Count	Mean	Variance	Std. Dev.	Std. Err
NS3/4-sp2	4	17.250	70.917	8.421	4.211
NS3/4-spNS3	4	6.250	4.917	2.217	1.109

20

EXAMPLE 11

To analyze whether administration of different NS3 containing compositions affected the elicitation of a cell-mediated immune response, mice were immunized with PBS, rNS3, irrelevant DNA or the NS3/4A construct, and tumor sizes were determined, as described above. Only the NS3/4A construct was able to elicit a T-cell response sufficient to cause a statistically significant reduction in tumor size (See TABLE 15). The example below describes experiments that were performed to determine whether the reduction in tumor size can be attributed to the generation of NS3-specific T-lymphocytes.

25

TABLE 15

Mouse ID	Immunogen	Dose (μ g)	Tumor cell line	Anti-NS3	Tumor growth	Maximum tumor size (mm)
1	NS3-pVAX	10	NS3/4A-SP2/0	<60	+	12.0
2	NS3-pVAX	10	NS3/4A-SP2/0	<60	+	20.0
3	NS3-pVAX	10	NS3/4A-SP2/0	60	+	18.0
4	NS3-pVAX	10	NS3/4A-SP2/0	<60	+	13.0
5	NS3-pVAX	10	NS3/4A-SP2/0	<60	+	17.0
Group mean				60	5/5	16.0 \pm 3.391
6	NS3-pVAX	100	NS3/4A-SP2/0	2160	+	10.0
7	NS3-pVAX	100	NS3/4A-SP2/0	<60	-	-
8	NS3-pVAX	100	NS3/4A-SP2/0	<60	-	-
9	NS3-pVAX	100	NS3/4A-SP2/0	360	-	-
10	NS3-pVAX	100	NS3/4A-SP2/0	<60	+	12.5
Group mean				1260	2/5	11.25 \pm 1.768
11	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	+	10.0
12	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	-	-
13	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	-	-
14	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	+	13.0
15	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	+	13.5
Group mean				<60	3/5	12.167 \pm 1.893
16	NS3/4A-pVAX	100	NS3/4A-SP2/0	60	+	10.0
17	NS3/4A-pVAX	100	NS3/4A-SP2/0	360	-	-
18	NS3/4A-pVAX	100	NS3/4A-SP2/0	2160	+	8.0
19	NS3/4A-pVAX	100	NS3/4A-SP2/0	2160	+	12.0
20	NS3/4A-pVAX	100	NS3/4A-SP2/0	2160	+	7.0
Group mean				1380	4/5	9.25 \pm 2.217
36	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	20.0
37	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	7.0
38	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	11.0
39	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	15.0
40	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	18.0
Group mean				<60	5/5	14.20 \pm 5.263
41	rNS3/CFA	20	NS3/4A-SP2/0	>466560	+	13.0
42	rNS3/CFA	20	NS3/4A-SP2/0	>466560	-	-
43	rNS3/CFA	20	NS3/4A-SP2/0	>466560	+	3.5
44	rNS3/CFA	20	NS3/4A-SP2/0	>466560	+	22.0
45	rNS3/CFA	20	NS3/4A-SP2/0	>466560	+	17.0
Group mean				466560	4/5	17.333 \pm 4.509
46	PBS	-	NS3/4A-SP2/0	<60	+	10.0
47	PBS	-	NS3/4A-SP2/0	<60	+	16.5
48	PBS	-	NS3/4A-SP2/0	60	+	15.0
49	PBS	-	NS3/4A-SP2/0	<60	+	21.0
50	PBS	-	NS3/4A-SP2/0	<60	+	15.0
51	PBS	-	NS3/4A-SP2/0	<60	-	-
Group mean				60	5/6	15.50 \pm 3.937

Note: Statistical analysis (StatView): Student's t-test on maximum tumor size. P-values < 0.05 are considered as significant.

Unpaired t-test for Largest Tumor size
 Grouping Variable: group
 Hypothesized Difference = 0

	Mean Diff.	DF	t-Value	P-Value
p17-sp3-4, NS3-100-sp3-4	2.950	5	.739	.4933
p17-sp3-4, NS3/4-10-sp3-4	2.033	6	.628	.5532
p17-sp3-4, NS3-10-sp3-4	-1.800	8	-.643	.5383
p17-sp3-4, NS3/4-100-sp3-4	4.950	7	1.742	.1250
p17-sp3-4, PBS-sp3-4	-1.300	8	-.442	.6700
p17-sp3-4, rNS3-sp3-4	-3.133	6	-.854	.4259
NS3-100-sp3-4, NS3/4-10-sp3-4	-.917	3	-.542	.6254
NS3-100-sp3-4, NS3-10-sp3-4	-4.750	5	-1.811	.1299
NS3-100-sp3-4, NS3/4-100-sp3-4	2.000	4	1.092	.3360
NS3-100-sp3-4, PBS-sp3-4	-4.250	5	-1.408	.2183
NS3-100-sp3-4, rNS3-sp3-4	-6.083	3	-1.744	.1795
NS3/4-10-sp3-4, NS3-10-sp3-4	-3.833	6	-1.763	.1283
NS3/4-10-sp3-4, NS3/4-100-sp3-4	2.917	5	1.824	.1277
NS3/4-10-sp3-4, PBS-sp3-4	-3.333	6	-1.344	.2274
NS3/4-10-sp3-4, rNS3-sp3-4	-5.167	4	-1.830	.1412
NS3-10-sp3-4, NS3/4-100-sp3-4	6.750	7	3.416	.0112
NS3-10-sp3-4, PBS-sp3-4	.500	8	.215	.8350
NS3-10-sp3-4, rNS3-sp3-4	-1.333	6	-.480	.6480
NS3/4-100-sp3-4, PBS-sp3-4	-6.250	7	-2.814	.0260
NS3/4-100-sp3-4, rNS3-sp3-4	-8.083	5	-3.179	.0246
PBS-sp3-4, rNS3-sp3-4	-1.833	6	-.607	.5662

EXAMPLE 12

- 5 To determine whether NS3-specific T-cells were elicited by the NS3/4A immunizations, an *in vitro* T-cell mediated tumor cell lysis assay was employed. The assay has been described in detail previously (Townsend et al., *J. Virol.* 71:3365 (1997)). Briefly, groups of five Balb/c mice were immunized three times with 100µg NS3/4A-pVAX *i.m.* Two weeks after the last injection the mice were sacrificed and splenocytes were harvested. Re-stimulation cultures with 3 x 10⁶
- 10 splenocytes and 3 x 10⁶ NS3/4A-SP2/0 cells were set. After five days, a standard Cr⁵¹-release assay was performed using NS3/4A-SP2/0 or SP2/0 cells as targets. Percent specific lysis was calculated as the ratio between lysis of NS3/4A-SP2/0 cells and lysis of SP2/0 cells. Only mice immunized with NS3/4A-pVAX displayed specific lysis over 10% in four out of five tested mice, using an effector to target ratio of 20:1 (See FIGURES 5A and B). Accordingly, mice immunized
- 15 with NS3/4A exhibited a reduction in cancer cell proliferation and/or NS3/4A caused the lysis of cancer cells. The section below describes several of the embodied HCV polypeptides in greater detail.

HCV peptides

The nucleic acids encoding the HCV peptides, described in the previous section, can be manipulated using conventional techniques in molecular biology so as to create recombinant constructs that express the HCV peptides. The embodied HCV peptides or derivatives thereof, include but are not limited to, those containing as a primary amino acid sequence all of the amino acid sequence substantially as depicted in the Sequence Listing (SEQ. ID. NOs.: 17, 29- 32 and 43-49) and fragments thereof at least four amino acids in length (e.g., SEQ. ID. NOs.: 25-27, and 33-42) including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. Preferred fragments of a sequence of SEQ. ID. NOs.: 17, 29- 32 and 43-49 are at least four amino acids and comprise amino acid sequence unique to the discovered NS3/4A peptide (SEQ. ID. NO.: 17) including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. The HCV peptides can be, for example, at least 12-15, 15-20, 20-25, 25-50, 50-100, 100-150, 150-250, 250-500 or 500-704 amino acids in length. Other fragments (e.g., SEQ. ID. NOs.: 25-27, and 33-42) are also aspects of the invention.

Embodiments of the invention also include HCV peptides that are substantially identical to those described above. That is, HCV peptides that have one or more amino acid residues within SEQ. ID. NO.: 17 and fragments thereof that are substituted by another amino acid of a similar polarity that acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence can be selected from other members of the class to which the amino acid belongs. For example, the non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine, and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. The aromatic amino acids include phenylalanine, tryptophan, and tyrosine.

The HCV peptides described herein can be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art such as those set forth by Merrifield et al., *J. Am. Chem. Soc.* 85:2149 (1964), Houghten et al., *Proc. Natl. Acad. Sci. USA*, 82:51:32 (1985), Stewart and Young (Solid phase peptide synthesis, Pierce Chem Co., Rockford, IL (1984), and Creighton, 1983, Proteins: Structures and Molecular Principles, W. H. Freeman & Co., N.Y. Such polypeptides can be synthesized with or without a methionine on the amino terminus. Chemically synthesized HCV peptides can be oxidized using methods set forth in these references to form disulfide bridges.

While the HCV peptides described herein can be chemically synthesized, it can be more effective to produce these polypeptides by recombinant DNA technology. Such methods can be used to construct expression vectors containing the HCV nucleotide sequences described above, for example, and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Alternatively, RNA capable of encoding HCV nucleotide sequences can be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in Oligonucleotide Synthesis, 1984, Gait, M. J. ed., IRL Press, Oxford. Accordingly, several embodiments concern cell lines that have been engineered to express the embodied HCV peptides. For example, some cells are made to express the HCV peptides of (SEQ. ID. NOs.: 17, 29- 32 and 43-49) or fragments of these molecules.

A variety of host-expression vector systems can be utilized to express the embodied HCV peptides. Suitable expression systems include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* or *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing HCV nucleotide sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the HCV nucleotide sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the HCV sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing HCV sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the HCV gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of HCV peptide or for raising antibodies to the HCV peptide, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified can be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.*, 2:1791 (1983), in which the HCV coding sequence can be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.*, 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.*, 264:5503-5509 (1989)); and the like. pGEX vectors can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general,

such fusion proteins are soluble and can be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

5 In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The HCV coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of an HCV gene coding sequence will result in inactivation of the
10 polyhedrin gene and production of non-occluded recombinant virus, (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (See e.g., Smith et al., *J. Virol.* 46: 584 (1983); and Smith, U.S. Pat. No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems can be utilized. In
15 cases where an adenovirus is used as an expression vector, the HCV nucleotide sequence of interest can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing
20 the HCV gene product in infected hosts. (See e.g., Logan & Shenk, *Proc. Natl. Acad. Sci. USA* 81:3655-3659 (1984)). Specific initiation signals can also be required for efficient translation of inserted HCV nucleotide sequences. These signals include the ATG initiation codon and adjacent sequences.

However, in cases where only a portion of the HCV coding sequence is inserted,
25 exogenous translational control signals, including, perhaps, the ATG initiation codon, can be provided. Furthermore, the initiation codon can be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer
30 elements, transcription terminators, etc. (See Bittner et al., *Methods in Enzymol.*, 153:516-544 (1987)).

In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products are important
35 for the function of the protein. Different host cells have characteristic and specific mechanisms for

the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product
5 can be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and WI38.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express the HCV peptides described above can be engineered. Rather than using expression vectors that contain viral origins of replication, host cells
10 can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells are allowed to grow for 1-2 days in an enriched media; and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably
15 integrate the plasmid into their chromosomes and grow to form foci which in turn are cloned and expanded into cell lines. This method is advantageously used to engineer cell lines which express the HCV gene product.

A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., *Cell* 11:223 (1977), hypoxanthine-guanine
20 phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA* 48:2026 (1962), and adenine phosphoribosyltransferase (Lowy, et al., *Cell* 22:817 (1980) genes can be employed in tk⁻, hgp⁺ or apr⁺ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler, et al.,
25 *Proc. Natl. Acad. Sci. USA* 77:3567 (1980); O'Hare, et al., *Proc. Natl. Acad. Sci. USA* 78:1527 (1981); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA* 78:2072 (1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., *J. Mol. Biol.* 150:1 (1981); and hyg^r, which confers resistance to hygromycin (Santerre, et al., *Gene* 30:147 (1984)).

Alternatively, any fusion protein can be readily purified by utilizing an antibody specific
30 for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines. (Janknecht, et al., *Proc. Natl. Acad. Sci. USA* 88: 8972-8976 (1991)). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from
35 cells infected with recombinant vaccinia virus are loaded onto Ni²⁺-nitriloacetic acid-agarose

columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers. The example below describes a method that was used to express the HCV peptides encoded by the embodied nucleic acids.

EXAMPLE 13

5 To characterize the NS3/4A fusion protein, and the truncated and mutated versions thereof, the vector constructs, described in *Example 6*, were transcribed and translated *in vitro*, and the resulting polypeptides were visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). *In vitro* transcription and translation were performed using the T7 coupled reticulocyte lysate system (Promega, Madison, WI) according to the manufacturer's
10 instructions. All *in vitro* translation reactions of the expression constructs were carried out at 30°C with ³⁵S-labeled methionine (Amersham International, Plc, Buckinghamshire, UK). The labeled proteins were separated on 12% SDS-PAGE gels and visualized by exposure to X-ray film (Hyper Film-MP, Amersham) for 6-18 hours.

The *in vitro* analysis revealed that all proteins were expressed to high amounts from their
15 respective expression constructs. The rNS3 construct (NS3-pVAX vector) produced a single peptide of approximately 61kDa, whereas, the TPT construct (NS3/4A-TPT-pVAX) and the RGT construct (NS3/4A-RGT-pVAX) produced a single polypeptide of approximately 67 kDa, which is identical to the molecular weight of the uncleaved NS3/4A peptide produced from the NS3/4A-pVAX construct. The cleaved product produced from the expressed NS3/4A peptide was
20 approximately 61 kDa, which was identical in size to the rNS3 produced from the NS3-pVAX vector. These results demonstrated that the expression constructs were functional, the NS3/4A construct was enzymatically active, the rNS3 produced a peptide of the predicted size, and the TPT and RGT mutations completely abolished cleavage at the NS3-NS4A junction.

25 The sequences, constructs, vectors, clones, and other materials comprising the embodied HCV nucleic acids and peptides can be in enriched or isolated form. As used herein, "enriched" means that the concentration of the material is at least about 2, 5, 10, 100, or 1000 times its natural concentration (for example), advantageously 0.01%, by weight, preferably at least about 0.1% by weight. Enriched preparations from about 0.5%, 1%, 5%, 10%, and 20% by weight are also
30 contemplated. The term "isolated" requires that the material be removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide present in a living animal is not isolated, but the same polynucleotide, separated from some or all of the coexisting materials in the natural system, is isolated. It is also advantageous that the sequences be in purified form. The term "purified" does not require absolute
35 purity; rather, it is intended as a relative definition. Isolated proteins have been conventionally

purified to electrophoretic homogeneity by Coomassie staining, for example. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated.

The HCV gene products described herein can also be expressed in plants, insects, and animals so as to create a transgenic organism. Desirable transgenic plant systems having an HCV peptide include *Arabidopsis*, maize, and *Chlamydomonas*. Desirable insect systems having an HCV peptide include, but are not limited to, *D. melanogaster* and *C. elegans*. Animals of any species, including, but not limited to, amphibians, reptiles, birds, mice, hamsters, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, dogs, cats, and non-human primates, e.g., baboons, monkeys, and chimpanzees can be used to generate transgenic animals having an embodied HCV molecule. These transgenic organisms desirably exhibit germline transfer of HCV peptides described herein.

Any technique known in the art is preferably used to introduce the HCV transgene into animals to produce the founder lines of transgenic animals or to knock out or replace existing HCV genes. Such techniques include, but are not limited to pronuclear microinjection (Hoppe, P. C. and Wagner, T. E., 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci., USA* 82:6148-6152 (1985); gene targeting in embryonic stem cells (Thompson et al., *Cell* 56:313-321 (1989); electroporation of embryos (Lo, *Mol. Cell. Biol.* 3:1803-1814 (1983); and sperm-mediated gene transfer (Lavitrano et al., *Cell* 57:717-723 (1989); see also Gordon, *Transgenic Animals, Intl. Rev. Cytol.* 115:171-229 (1989). The section below describes the manufacture of antibodies that interact with the HCV peptides described herein.

Anti-HCV antibodies

Following synthesis or expression and isolation or purification of the HCV peptides, the isolated or purified peptide can be used to generate antibodies. Depending on the context, the term "antibodies" can encompass polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library. Antibodies that recognize the HCV peptides have many uses including, but not limited to, biotechnological applications, therapeutic/prophylactic applications, and diagnostic applications.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, and humans etc. can be immunized by injection with an HCV peptide. Depending on the host species, various adjuvants can be used to increase immunological response. Such adjuvants include, but are not limited to, ribavirin, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (*Bacillus Calmette-Guerin*) and *Corynebacterium parvum* are also potentially useful adjuvants.

Peptides used to induce specific antibodies can have an amino acid sequence consisting of at least four amino acids, and preferably at least 10 to 15 amino acids. By one approach, short stretches of amino acids encoding fragments of NS3/4A are fused with those of another protein such as keyhole limpet hemocyanin such that an antibody is produced against the chimeric molecule. Additionally, a composition comprising ribavirin and NS3/4A (SEQ. ID. NO.: 17), a fragment thereof at least 4, 6, 8, 10, 12, 15, or 20 amino acids in length, or a nucleic acid encoding one or more of these molecules is administered to an animal. While antibodies capable of specifically recognizing HCV can be generated by injecting synthetic 3-mer, 10-mer, and 15-mer peptides that correspond to an HCV peptide into mice, a more diverse set of antibodies can be generated by using recombinant HCV peptides, prepared as described above.

To generate antibodies to an HCV peptide, substantially pure peptide is isolated from a transfected or transformed cell. The concentration of the peptide in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the peptide of interest can then be prepared as follows:

Monoclonal antibodies to an HCV peptide can be prepared using any technique that provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique originally described by Koehler and Milstein (*Nature* 256:495-497 (1975)), the human B-cell hybridoma technique (Kosbor et al. *Immunol Today* 4:72 (1983); Cote et al *Proc Natl Acad Sci* 80:2026-2030 (1983)), and the EBV-hybridoma technique Cole et al. Monoclonal Antibodies and Cancer Therapy, Alan R. Liss Inc, New York N.Y., pp 77-96 (1985). In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used. (Morrison et al. *Proc Natl Acad Sci* 81:6851-6855 (1984); Neuberger et al. *Nature* 312:604-608(1984); Takeda et al. *Nature* 314:452-454(1985). Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce HCV-specific single chain antibodies. Antibodies can also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi et al., *Proc Natl Acad Sci* 86: 3833-3837 (1989), and Winter G. and Milstein C; *Nature* 349:293-299 (1991).

Antibody fragments that contain specific binding sites for an HCV peptide can also be generated. For example, such fragments include, but are not limited to, the F(ab')₂ fragments that can be produced by pepsin digestion of the antibody molecule and the Fab fragments that can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression

libraries can be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (Huse W. D. et al. *Science* 256:1275-1281 (1989)).

By one approach, monoclonal antibodies to an HCV peptide are made as follows. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein or peptides derived therefrom over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused in the presence of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, E., *Meth. Enzymol.* 70:419 (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. Basic Methods in Molecular Biology Elsevier, New York. Section 21-2.

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein or peptides derived therefrom described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than others and can require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. et al. *J. Clin. Endocrinol. Metab.* 33:988-991 (1971).

Booster injections are given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. et al., Chap. 19 in: Handbook of Experimental Immunology D. Wier (ed) Blackwell (1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 μ M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: Manual of Clinical Immunology, 2d Ed. (Rose and Friedman, Eds.) Amer. Soc. For Microbiol., Washington, D.C. (1980). Antibody preparations prepared according to either protocol are useful in quantitative immunoassays that determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or

qualitatively (e.g., in diagnostic embodiments that identify the presence of HCV in biological samples). The section below describes some of the diagnostic embodiments in greater detail.

Diagnostic embodiments

Generally, the embodied diagnostics are classified according to whether a nucleic acid or protein-based assay is used. Some diagnostic assays detect the presence or absence of an embodied HCV nucleic acid sequence in a sample obtained from a patient, whereas, other assays seek to identify whether an embodied HCV peptide is present in a biological sample obtained from a patient. Additionally, the manufacture of kits that incorporate the reagents and methods described herein that allow for the rapid detection and identification of HCV are also embodied. These diagnostic kits can include, for example, an embodied nucleic acid probe or antibody, which specifically detects HCV. The detection component of these kits will typically be supplied in combination with one or more of the following reagents. A support capable of absorbing or otherwise binding DNA, RNA, or protein will often be supplied. Available supports include membranes of nitrocellulose, nylon or derivatized nylon that can be characterized by bearing an array of positively charged substituents. One or more restriction enzymes, control reagents, buffers, amplification enzymes, and non-human polynucleotides like calf-thymus or salmon-sperm DNA can be supplied in these kits.

Useful nucleic acid-based diagnostics include, but are not limited to, direct DNA sequencing, Southern Blot analysis, dot blot analysis, nucleic acid amplification, and combinations of these approaches. The starting point for these analysis is isolated or purified nucleic acid from a biological sample obtained from a patient suspected of contracting HCV or a patient at risk of contracting HCV. The nucleic acid is extracted from the sample and can be amplified by RT-PCR and/or DNA amplification using primers that correspond to regions flanking the embodied HCV nucleic acid sequences (e.g., NS3/4A (SEQ. ID. NO.: 16)).

In some embodiments, nucleic acid probes that specifically hybridize with HCV sequences are attached to a support in an ordered array, wherein the nucleic acid probes are attached to distinct regions of the support that do not overlap with each other. Preferably, such an ordered array is designed to be "addressable" where the distinct locations of the probe are recorded and can be accessed as part of an assay procedure. These probes are joined to a support in different known locations. The knowledge of the precise location of each nucleic acid probe makes these "addressable" arrays particularly useful in binding assays. The nucleic acids from a preparation of several biological samples are then labeled by conventional approaches (e.g., radioactivity or fluorescence) and the labeled samples are applied to the array under conditions that permit hybridization.

If a nucleic acid in the samples hybridizes to a probe on the array, then a signal will be detected at a position on the support that corresponds to the location of the hybrid. Since the

identity of each labeled sample is known and the region of the support on which the labeled sample was applied is known, an identification of the presence of the polymorphic variant can be rapidly determined. These approaches are easily automated using technology known to those of skill in the art of high throughput diagnostic or detection analysis.

5 Additionally, an opposite approach to that presented above can be employed. Nucleic acids present in biological samples can be disposed on a support so as to create an addressable array. Preferably, the samples are disposed on the support at known positions that do not overlap. The presence of HCV nucleic acids in each sample is determined by applying labeled nucleic acid probes that complement nucleic acids, which encode HCV peptides, at locations on the array that
10 correspond to the positions at which the biological samples were disposed. Because the identity of the biological sample and its position on the array is known, the identification of a patient that has been infected with HCV can be rapidly determined. These approaches are also easily automated using technology known to those of skill in the art of high throughput diagnostic analysis.

 Any addressable array technology known in the art can be employed. One particular
15 embodiment of polynucleotide arrays is known as Genechips™, and has been generally described in US Patent 5,143,854; PCT publications WO 90/15070 and 92/10092. These arrays are generally produced using mechanical synthesis methods or light directed synthesis methods, which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis. (Fodor et al., *Science*, 251:767-777, (1991)). The immobilization of arrays of oligonucleotides on
20 solid supports has been rendered possible by the development of a technology generally identified as "Very Large Scale Immobilized Polymer Synthesis" (VLSPIS™) in which, typically, probes are immobilized in a high density array on a solid surface of a chip. Examples of VLSPIS™ technologies are provided in US Patents 5,143,854 and 5,412,087 and in PCT Publications WO 90/15070, WO 92/10092 and WO 95/11995, which describe methods for forming oligonucleotide
25 arrays through techniques such as light-directed synthesis techniques. In designing strategies aimed at providing arrays of nucleotides immobilized on solid supports, further presentation strategies were developed to order and display the oligonucleotide arrays on the chips in an attempt to maximize hybridization patterns and diagnostic information. Examples of such presentation strategies are disclosed in PCT Publications WO 94/12305, WO 94/11530, WO 97/29212, and WO
30 97/31256.

 A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid assays. There are several ways to produce labeled nucleic acids for hybridization or PCR including, but not limited to, oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, a nucleic acid encoding
35 an HCV peptide can be cloned into a vector for the production of an mRNA probe. Such vectors

are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labeled nucleotides. A number of companies such as Pharmacia Biotech (Piscataway N.J.), Promega (Madison Wis.), and U.S. Biochemical Corp (Cleveland Ohio) supply commercial kits and protocols for these procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as, substrates, cofactors, inhibitors, magnetic particles and the like.

The presence of an HCV peptide in a protein sample obtained from a patient can also be detected by using conventional assays and the embodiments described herein. For example, antibodies that are immunoreactive with the disclosed HCV peptides can be used to screen biological samples for the presence of HCV infection. In preferred embodiments, antibodies that are reactive to the embodied HCV peptides are used to immunoprecipitate the disclosed HCV peptides from biological samples or are used to react with proteins obtained from a biological sample on Western or Immunoblots. Favored diagnostic embodiments also include enzyme-linked immunosorbant assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies specific for the disclosed HCV peptides. Exemplary sandwich assays are described by David et al., in U.S. Patent Nos. 4,376,110 and 4,486,530. Other embodiments employ aspects of the immune-strip technology disclosed in U.S. Patent Nos. 5,290,678; 5,604,105; 5,710,008; 5,744,358; and 5,747,274.

In another preferred protein-based diagnostic, the antibodies described herein are attached to a support in an ordered array, wherein a plurality of antibodies are attached to distinct regions of the support that do not overlap with each other. As with the nucleic acid-based arrays, the protein-based arrays are ordered arrays that are designed to be "addressable" such that the distinct locations are recorded and can be accessed as part of an assay procedure. These probes are joined to a support in different known locations. The knowledge of the precise location of each probe makes these "addressable" arrays particularly useful in binding assays. For example, an addressable array can comprise a support having several regions to which are joined a plurality of antibody probes that specifically recognize HCV peptides present in a biological sample and differentiate the isotype of HCV identified herein.

By one approach, proteins are obtained from biological samples and are then labeled by conventional approaches (e.g., radioactivity, colorimetrically, or fluorescently). The labeled samples are then applied to the array under conditions that permit binding. If a protein in the sample binds to an antibody probe on the array, then a signal will be detected at a position on the support that corresponds to the location of the antibody-protein complex. Since the identity of

each labeled sample is known and the region of the support on which the labeled sample was applied is known, an identification of the presence, concentration, and/or expression level can be rapidly determined. That is, by employing labeled standards of a known concentration of HCV peptide, an investigator can accurately determine the protein concentration of the particular peptide in a tested sample and can also assess the expression level of the HCV peptide. Conventional methods in densitometry can also be used to more accurately determine the concentration or expression level of the HCV peptide. These approaches are easily automated using technology known to those of skill in the art of high throughput diagnostic analysis.

In another embodiment, an opposite approach to that presented above can be employed. Proteins present in biological samples can be disposed on a support so as to create an addressable array. Preferably, the protein samples are disposed on the support at known positions that do not overlap. The presence of an HCV peptide in each sample is then determined by applying labeled antibody probes that recognize epitopes specific for the HCV peptide. Because the identity of the biological sample and its position on the array is known, an identification of the presence, concentration, and/or expression level of an HCV peptide can be rapidly determined.

That is, by employing labeled standards of a known concentration of HCV peptide, an investigator can accurately determine the concentration of peptide in a sample and from this information can assess the expression level of the peptide. Conventional methods in densitometry can also be used to more accurately determine the concentration or expression level of the HCV peptide. These approaches are also easily automated using technology known to those of skill in the art of high throughput diagnostic analysis. As detailed above, any addressable array technology known in the art can be employed. The section below describes some of the compositions that can have one or more of the embodied HCV nucleic acids or HCV peptides.

Compositions comprising the embodied HCV nucleic acids or peptides

Some embodiments contain at least one of the HCV nucleic acids or peptides joined to a support. Preferably, these supports are manufactured so as to create a multimeric agent. These multimeric agents provide the HCV peptide or nucleic acid in such a form or in such a way that a sufficient affinity to the molecule is achieved. A multimeric agent having an HCV nucleic acid or peptide can be obtained by joining the desired molecule to a macromolecular support. A "support" can be termed a carrier, a protein, a resin, a cell membrane, or any macromolecular structure used to join or immobilize such molecules. Solid supports include, but are not limited to, the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, animal cells, Duracyte®, artificial cells, and others. An HCV nucleic acid or peptide can also be joined to inorganic carriers, such as silicon

oxide material (e.g., silica gel, zeolite, diatomaceous earth or aminated glass) by, for example, a covalent linkage through a hydroxy, carboxy or amino group and a reactive group on the carrier.

In several multimeric agents, the macromolecular support has a hydrophobic surface that interacts with a portion of the HCV nucleic acid or peptide by a hydrophobic non-covalent interaction. In some cases, the hydrophobic surface of the support is a polymer such as plastic or any other polymer in which hydrophobic groups have been linked such as polystyrene, polyethylene or polyvinyl. Additionally, HCV nucleic acid or peptide can be covalently bound to carriers including proteins and oligo/polysaccharides (e.g. cellulose, starch, glycogen, chitosane or aminated sepharose). In these later multimeric agents, a reactive group on the molecule, such as a hydroxy or an amino group, is used to join to a reactive group on the carrier so as to create the covalent bond. Additional multimeric agents comprise a support that has other reactive groups that are chemically activated so as to attach the HCV nucleic acid or peptide. For example, cyanogen bromide activated matrices, epoxy activated matrices, thio and thiopropyl gels, nitrophenyl chloroformate and N-hydroxy succinimide chloroformate linkages, or oxirane acrylic supports are used. (Sigma).

Carriers for use in the body, (i.e. for prophylactic or therapeutic applications) are desirably physiological, non-toxic and preferably, non-immunoresponsive. Suitable carriers for use in the body include poly-L-lysine, poly-D, L-alanine, liposomes, and Chromosorb® (Johns-Manville Products, Denver Co.). Ligand conjugated Chromosorb® (Synsorb-Pk) has been tested in humans for the prevention of hemolytic-uremic syndrome and was reported as not presenting adverse reactions. (*Armstrong et al. J. Infectious Diseases 171:1042-1045 (1995)*). For some embodiments, a "naked" carrier (i.e., lacking an attached HCV nucleic acid or peptide) that has the capacity to attach an HCV nucleic acid or peptide in the body of a organism is administered. By this approach, a "prodrug-type" therapy is envisioned in which the naked carrier is administered separately from the HCV nucleic acid or peptide and, once both are in the body of the organism, the carrier and the HCV nucleic acid or peptide are assembled into a multimeric complex.

The insertion of linkers, such as linkers (e.g., "λ linkers" engineered to resemble the flexible regions of λ phage) of an appropriate length between the HCV nucleic acid or peptide and the support are also contemplated so as to encourage greater flexibility of the HCV peptide, hybrid, or binding partner and thereby overcome any steric hindrance that can be presented by the support. The determination of an appropriate length of linker that allows for an optimal cellular response or lack thereof, can be determined by screening the HCV nucleic acid or peptide with varying linkers in the assays detailed in the present disclosure.

A composite support comprising more than one type of HCV nucleic acid or peptide is also envisioned. A "composite support" can be a carrier, a resin, or any macromolecular structure used

to attach or immobilize two or more different HCV nucleic acids or peptides. As above, the insertion of linkers, such as λ linkers, of an appropriate length between the HCV nucleic acid or peptide and the support is also contemplated so as to encourage greater flexibility in the molecule and thereby overcome any steric hindrance that can occur. The determination of an appropriate length of linker that allows for an optimal cellular response or lack thereof, can be determined by screening the HCV nucleic acid or peptide with varying linkers in the assays detailed in the present disclosure.

In other embodiments, the multimeric and composite supports discussed above can have attached multimerized HCV nucleic acids or peptides so as to create a "multimerized-multimeric support" and a "multimerized-composite support", respectively. A multimerized ligand can, for example, be obtained by coupling two or more HCV nucleic acids or peptides in tandem using conventional techniques in molecular biology. The multimerized form of the HCV nucleic acid or peptide can be advantageous for many applications because of the ability to obtain an agent with a higher affinity, for example. The incorporation of linkers or spacers, such as flexible λ linkers, between the individual domains that make-up the multimerized agent can also be advantageous for some embodiments. The insertion of λ linkers of an appropriate length between protein binding domains, for example, can encourage greater flexibility in the molecule and can overcome steric hindrance. Similarly, the insertion of linkers between the multimerized HCV nucleic acid or peptide and the support can encourage greater flexibility and limit steric hindrance presented by the support. The determination of an appropriate length of linker can be determined by screening the HCV nucleic acids or peptides in the assays detailed in this disclosure.

Embodiments of the invention also include genetic vaccines, as described above. Preferably these compositions contain ribavirin and a nucleic acid encoding NS3/4A (SEQ. ID. NO.: 17), NS3 (SEQ. ID. NO.: 29), or a mutant (e.g., SEQ. ID. NOs.: 30 - 32 and 43-49) or a fragment thereof (e.g., SEQ. ID. NOs.: 25-27, and 33-42). The following example describes the preparation of a genetic vaccine suitable for use in humans.

EXAMPLE 14

An HCV expression plasmid is designed to express the NS3/4A peptide. The NS3/4A coding sequence of NS3/4A-pVAX is removed by digestion with *EcoRI* and *XbaI*, and the isolated fragment is inserted into plasmid A so that it is under the transcriptional control of the CMV promoter and the RSV enhancer element. (See U.S. Pat. No. 6,235,888 to Pachuk, et al.). Plasmid backbone A is 3969 base pairs in length; it contains a PBR origin of replication for replicating in *E. coli* and a kanamycin resistance gene. Inserts such as the NS3/4A, are cloned into a polylinker region, which places the insert between and operably linked to the promoter and polyadenylation signal. Transcription of the cloned inserts is under the control of the CMV promoter and the RSV

enhancer elements. A polyadenylation signal is provided by the presence of an SV40 poly A signal situated just 3' of the cloning site. An NS3/4A containing vaccine composition is then made by mixing 500µg of the rNS3/4A construct with 1mg of ribavirin.

Said vaccine composition can be used to raise antibodies in a mammal (e.g., mice or rabbits) or can be injected intramuscularly into a human so as to raise antibodies, preferably a human that is chronically infected with the HCV virus. The recipient preferably receives three immunization boosts of the mixture at 4-week intervals, as well. By the third boost, the titer of antibody specific for HCV will be significantly increased. Additionally, at this time, said subject will experience an enhanced antibody and T-cell mediated immune response against NS3, as evidenced by an increased fraction of NS3 specific antibodies as detected by EIA, and a reduction in viral load as detected by RT-PCR.

Embodiments also include NS3/4A fusion proteins or nucleic acids encoding these molecules. For instance, production and purification of recombinant protein may be facilitated by the addition of auxiliary amino acids to form a "tag". Such tags include, but are not limited to, His-6, Flag, Myc and GST. The tags may be added to the C-terminus, N-terminus, or within the NS3/4A amino acid sequence. Further embodiments include NS3/4A fusion proteins with amino or carboxy terminal truncations, or internal deletions, or with additional polypeptide sequences added to the amino or carboxy terminal ends, or added internally. Other embodiments include NS3/4A fusion proteins, or truncated or mutated versions thereof, where the residues of the NS3/4A proteolytic cleavage site have been substituted. Such substitutions include, but are not limited to, sequences where the P1' site is a Ser, Gly, or Pro, or the P1 position is an Arg, or where the P8 to P4' sequence is Ser-Ala-Asp-Leu-Glu-Val-Val-Thr-Ser-Thr-Trp-Val (SEQ. ID. NO.: 28).

Other embodiments concern an immunogen comprising the NS3/4A fusion protein, or a truncated or modified version thereof, capable of eliciting an enhanced immune response against NS3. The immunogen can be provided in a substantially purified form, which means that the immunogen has been rendered substantially free of other proteins, lipids, carbohydrates or other compounds with which it naturally associates. Embodiments also include vaccine compositions comprising the NS3/4A fusion protein (SEQ. ID. NO.: 17), or a truncated or mutated version thereof (e.g., SEQ. ID. NOS.: 29- 32 and 43-49) or a fragment thereof (e.g., SEQ. ID. NOS.: 25- 27, and 33-42), and an adjuvant, such as ribavirin. The following example describes one approach to prepare a vaccine composition comprising the NS3/4A fusion protein and an adjuvant.

Example 15

To generate a tagged NS3/4A construct, the NS3/4A coding sequence of NS3/4A-pVAX is removed by digestion with *EcoRI* and *XbaI*, and the isolated fragment is inserted into an Xpress vector (Invitrogen). The Xpress vector allows for the production of a recombinant fusion protein having a short N-terminal leader peptide that has a high affinity for divalent cations. Using a nickel-chelating resin (Invitrogen), the recombinant protein can be purified in one step and the leader can be subsequently removed by cleavage with enterokinase. A preferred vector is the pBlueBacHis2 Xpress. The pBlueBacHis2 Xpress vector is a Baculovirus expression vector containing a multiple cloning site, an ampicillin resistance gene, and a *lac z* gene. Accordingly, the digested amplification fragment is cloned into the pBlueBacHis2 Xpress vector and SF9 cells are infected. The expression protein is then isolated or purified according to the manufacturer's instructions. An NS3/4A containing vaccine composition is then made by mixing 100µg of the rNS3/4A with 1mg of ribavirin.

Said vaccine composition can be used to raise antibodies in a mammal (e.g., mice or rabbits) or can be injected intramuscularly into a human so as to raise antibodies, preferably a human that is chronically infected with the HCV virus. The recipient preferably receives three immunization boosts of the mixture at 4-week intervals. By the third boost, the titer of antibody specific for HCV will be significantly increased. Additionally, at this time, said subject will experience an enhanced antibody and T-cell mediated immune response against NS3, as evidenced by an increased fraction of NS3 specific antibodies as detected by EIA, and a reduction in viral load as detected by RT-PCR. The section below provides more explanation concerning the methods of using the compositions described herein.

Methods of using compositions comprising ribavirin and an antigen

Routes of administration of the vaccines described herein include, but are not limited to, transdermal, parenteral, gastrointestinal, transbronchial, and transalveolar. Transdermal administration can be accomplished by application of a cream, rinse, gel, or other compounds capable of allowing ribavirin and antigen to penetrate the skin. Parenteral routes of administration include, but are not limited to, electrical or direct injection such as direct injection into a central venous line, intravenous, intramuscular, intraperitoneal, intradermal, or subcutaneous injection. Gastrointestinal routes of administration include, but are not limited to, ingestion and rectal. Transbronchial and transalveolar routes of administration include, but are not limited to, inhalation, either via the mouth or intranasally.

Compositions having ribavirin and an antigen that are suitable for transdermal administration include, but are not limited to, pharmaceutically acceptable suspensions, oils, creams, and ointments applied directly to the skin or incorporated into a protective carrier such as a

transdermal device ("transdermal patch"). Examples of suitable creams, ointments, etc. can be found, for instance, in the Physician's Desk Reference. Examples of suitable transdermal devices are described, for instance, in U.S. Patent No. 4,818,540 issued April 4, 1989 to Chinen, et al.

5 Compositions having ribavirin and an antigen that are suitable for parenteral administration include, but are not limited to, pharmaceutically acceptable sterile isotonic solutions. Such solutions include, but are not limited to, saline, phosphate buffered saline and oil preparations for injection into a central venous line, intravenous, intramuscular, intraperitoneal, intradermal, or subcutaneous injection.

10 Compositions having ribavirin and an antigen that are suitable for transbronchial and transalveolar administration include, but not limited to, various types of aerosols for inhalation. Devices suitable for transbronchial and transalveolar administration of these are also embodiments. Such devices include, but are not limited to, atomizers and vaporizers. Many forms of currently available atomizers and vaporizers can be readily adapted to deliver vaccines having ribavirin and an antigen.

15 Compositions having ribavirin and an antigen that are suitable for gastrointestinal administration include, but not limited to, pharmaceutically acceptable powders, pills or liquids for ingestion and suppositories for rectal administration.

20 The gene constructs described herein, in particular, may be administered by means including, but not limited to, traditional syringes, needleless injection devices, or "microprojectile bombardment gene guns". Alternatively, the genetic vaccine may be introduced by various means into cells that are removed from the individual. Such means include, for example, *ex vivo* transfection, electroporation, microinjection and microprojectile bombardment. After the gene construct is taken up by the cells, they are reimplanted into the individual. It is contemplated that otherwise non-immunogenic cells that have gene constructs incorporated therein can be implanted
25 into the individual even if the vaccinated cells were originally taken from another individual.

According to some embodiments, the gene construct is administered to an individual using a needleless injection device. According to some embodiments, the gene construct is simultaneously administered to an individual intradermally, subcutaneously and intramuscularly using a needleless injection device. Needleless injection devices are well known and widely
30 available. One having ordinary skill in the art can, following the teachings herein, use needleless injection devices to deliver genetic material to cells of an individual. Needleless injection devices are well suited to deliver genetic material to all tissue. They are particularly useful to deliver genetic material to skin and muscle cells. In some embodiments, a needleless injection device may be used to propel a liquid that contains DNA molecules toward the surface of the individual's skin.
35 The liquid is propelled at a sufficient velocity such that upon impact with the skin the liquid

penetrates the surface of the skin, permeates the skin and muscle tissue therebeneath. Thus, the genetic material is simultaneously administered intradermally, subcutaneously and intramuscularly. In some embodiments, a needleless injection device may be used to deliver genetic material to tissue of other organs in order to introduce a nucleic acid molecule to cells of
5 that organ.

The vaccines containing ribavirin and an antigen can be used to treat and prevent a vast spectrum of diseases and can enhance the immune response of an animal to an antigen. As one of skill in the art will appreciate, conventional vaccines have been administered to subjects in need of treatment or prevention of bacterial diseases, viral diseases, fungal diseases, and cancer. Because
10 the vaccines described herein include conventional vaccines, which have been modified by the addition of ribavirin, the methods described herein include the treatment and prevention of a disease using a vaccine that comprises an antigen and ribavirin.

Preferred embodiments concern methods of treating or preventing hepatitis infection. In these embodiments, an animal in need is provided a hepatitis antigen (e.g., a peptide antigen or
15 nucleic acid-based antigen) and an amount of ribavirin sufficient to exhibit an adjuvant activity in said animal. Accordingly, an animal can be identified as one in need by using currently available diagnostic testing or clinical evaluation. The range of hepatitis viral antigens that can be used with these embodiments is diverse. Preferred hepatitis viral antigens include an HBV antigen, an HAV antigen, an HCV antigen, nucleic acids encoding these antigens, or any combination thereof.
20 Highly preferred embodiments include an HBV antigen selected from the group consisting of hepatitis B surface antigen (HBsAg), hepatitis core antigen (HBcAg), and hepatitis E antigen (HBeAg), in particular, the peptide and nucleic acid-based antigens described *supra*. The ribavirin and antigen can be provided separately or in combination, and other adjuvants (e.g., oil, alum, or other agents that enhance an immune response) can also be provided to the animal in need. Thus,
25 preferred embodiments include methods of treating or preventing hepatitis in an animal (e.g., HBV) by identifying an infected animal or an animal at risk of infection and providing said animal a hepatitis antigen (e.g., HBsAg, HBcAg, and HBeAg) and an amount of ribavirin sufficient to exhibit adjuvant activity.

Other embodiments include methods of enhancing an immune response to an antigen by
30 providing an animal in need with an amount of ribavirin that is effective to enhance said immune response. In these embodiments, an animal in need of an enhanced immune response to an antigen is identified by using currently available diagnostic testing or clinical evaluation. Oftentimes these individuals will be suffering from a disease (e.g., bacterial, fungal, mold, viral, or cancer) or are at risk from contracting the disease. However, an animal in need of an enhanced immune response
35 can be an animal that has been poisoned (e.g., bit by a poisonous insect or animal) or that has been

exposed to a toxin or other toxic compound. Once identified, these animals are provided an appropriate antigen and an amount of ribavirin effective to enhance an immune response in the animal.

5 As above, the hepatitis viral antigens that can be used with these embodiments include, but are not limited to, an HBV antigen, an HAV antigen, an HCV antigen, a nucleic acid encoding these molecules, or any combination thereof. Highly preferred embodiments include an HBV antigen selected from the group consisting of hepatitis B surface antigen (HBsAg), hepatitis core antigen (HBcAg), and hepatitis E antigen (HBeAg), in particular, the peptide and nucleic acid-based antigens described *supra*. The ribavirin and antigen can be provided separately or in
10 combination, and other adjuvants (e.g., oil, alum, or other agents that enhance an immune response) can also be provided to the animal in need. Thus, preferred embodiments include methods of enhancing an immune response to a hepatitis antigen (e.g., HBV) by identifying an animal in need and providing the animal a hepatitis antigen (e.g., HBsAg, HBcAg, and HBeAg) and an amount of ribavirin that is effective to enhance an immune response in the animal.

15 By one approach, for example, an uninfected individual is provided with the above mentioned vaccine compositions in an amount sufficient to elicit a cellular and humoral immune response to NS3 so as to protect said individual from becoming infected with HCV. In another embodiment, an HCV-infected individual is identified and provided with a vaccine composition comprising ribavirin and NS3 in an amount sufficient to enhance the cellular and humoral immune
20 response against NS3 so as to reduce or eliminate the HCV infection. Such individual may be in the chronic or acute phase of the infection. In yet another embodiment, an HCV-infected individual suffering from HCC is provided with a composition comprising ribavirin and the NS3/4A fusion gene in an amount sufficient to elicit a cellular and humoral immune response against NS3-expressing tumor cells.

25 Although the invention has been described with reference to embodiments and examples, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

WHAT IS CLAIMED IS:

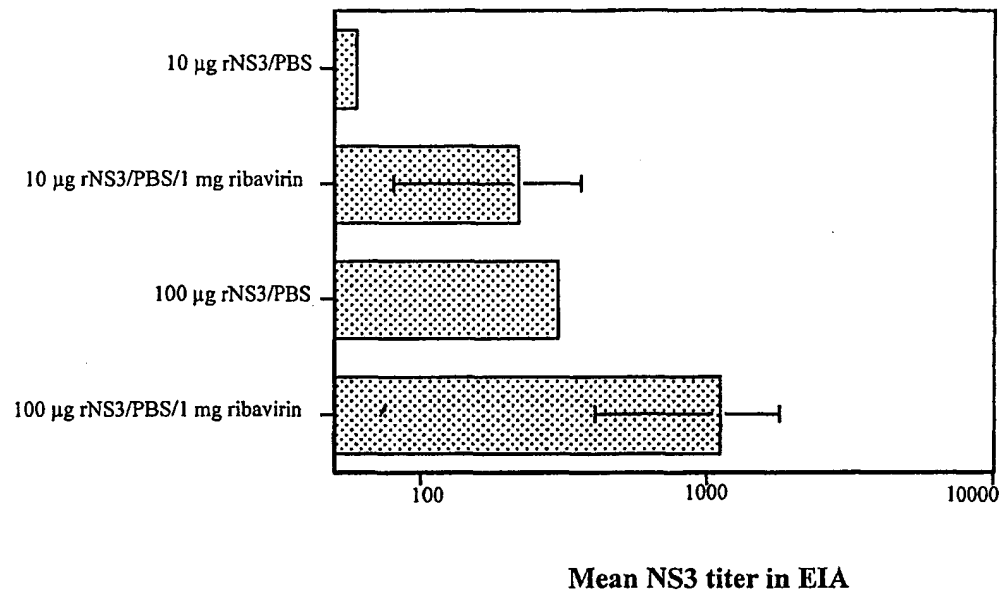
1. A composition comprising ribavirin and the nucleic acid of SEQ. ID. NO.: 16.
2. A composition comprising Ribavirin and the peptide of SEQ. ID. NO.: 17.
3. A composition comprising Ribavirin and the nucleic acid of SEQ. ID. NO.: 13 or a
5 fragment thereof at least 18 consecutive nucleotides in length.
4. A composition comprising ribavirin and the peptide of SEQ. ID. NO.: 1 or a
fragment thereof at least 6 consecutive amino acids in length.
5. A composition comprising ribavirin and an antigen.
6. The composition of Claim 5, wherein said antigen is a nucleic acid.
- 10 7. The composition of Claim 5, wherein said antigen is a peptide.
8. The composition of Claim 6, wherein said nucleic acid is derived from a virus
selected from the group consisting of hepatitis A virus (HAV), hepatitis B virus (HBV), and
hepatitis C virus (HCV).
9. The composition of Claim 7, wherein said peptide is derived from a virus selected
15 from the group consisting of hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C
virus (HCV).
10. The composition of Claim 5, wherein said antigen is a nucleic acid or a peptide
corresponding to an antigen selected from the group consisting of hepatitis B surface antigen
(HBsAg), hepatitis core antigen (HBcAg), and hepatitis E antigen (HEAg).
- 20 11. The composition of Claim 7, wherein said peptide comprises at least three
consecutive amino acids of a sequence selected from the group consisting of SEQ. ID. NOS.: 1-12.
12. The composition of Claim 6, wherein said nucleic acid comprises at least 9
consecutive nucleotides of a sequence selected from the group consisting of SEQ. ID. NOS.: 13-
15.
- 25 13. A method of enhancing an immune response to a hepatitis C antigen comprising:
identifying an animal in need of an enhanced immune response to a hepatitis C
antigen; and
providing to said animal a composition comprising ribavirin and the nucleic acid
of SEQ. ID. NO.: 16.
- 30 14. A method of enhancing an immune response to a hepatitis C antigen comprising:
identifying an animal in need of an enhanced immune response to a hepatitis C
antigen; and
providing to said animal a composition comprising ribavirin and the peptide of
SEQ. ID. NO.: 17.

35

15. A method of enhancing an immune response to a hepatitis C antigen comprising:
identifying an animal in need of an enhanced immune response to a hepatitis C
antigen; and
providing to said animal a composition comprising ribavirin and the nucleic acid
of SEQ. ID. NO.: 13 or a fragment thereof at least 18 consecutive nucleotides in length.
16. A method of enhancing an immune response to a hepatitis C antigen comprising:
identifying an animal in need of an enhanced immune response to a hepatitis C
antigen; and
providing to said animal a composition comprising ribavirin and the peptide of
SEQ. ID. NO.: 1 or a fragment thereof at least 6 consecutive amino acids in length.
17. A method of making a vaccine comprising:
providing ribavirin;
providing the nucleic acid of SEQ. ID. NO.: 16; and
mixing said ribavirin and said nucleic acid so as to formulate said vaccine.
18. A method of making a vaccine comprising:
providing ribavirin;
providing the peptide of SEQ. ID. NO.: 17; and
mixing said ribavirin and said peptide so as to formulate said vaccine.
19. A method of making a vaccine comprising:
providing ribavirin;
providing the nucleic acid of SEQ. ID. NO.: 13 or a fragment thereof at least at
least 18 consecutive nucleotides in length; and
mixing said ribavirin and said nucleic acid so as to formulate said vaccine.
20. A method of making a vaccine comprising:
providing ribavirin;
providing the nucleic acid of SEQ. ID. NO.: 1 or a fragment thereof at least 6
consecutive amino acids in length; and
mixing said ribavirin and said nucleic acid so as to formulate said vaccine.

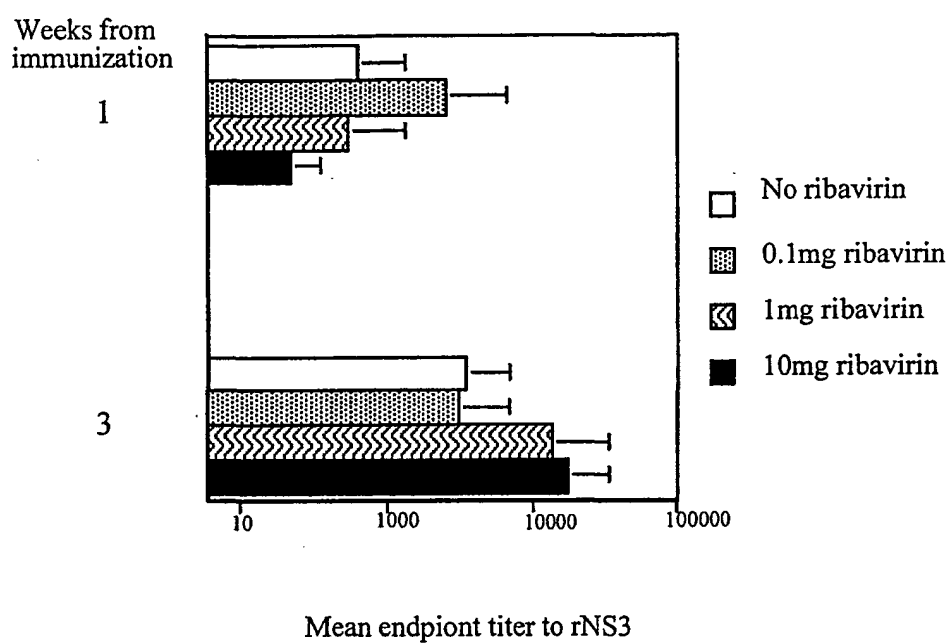
1/5

FIGURE 1



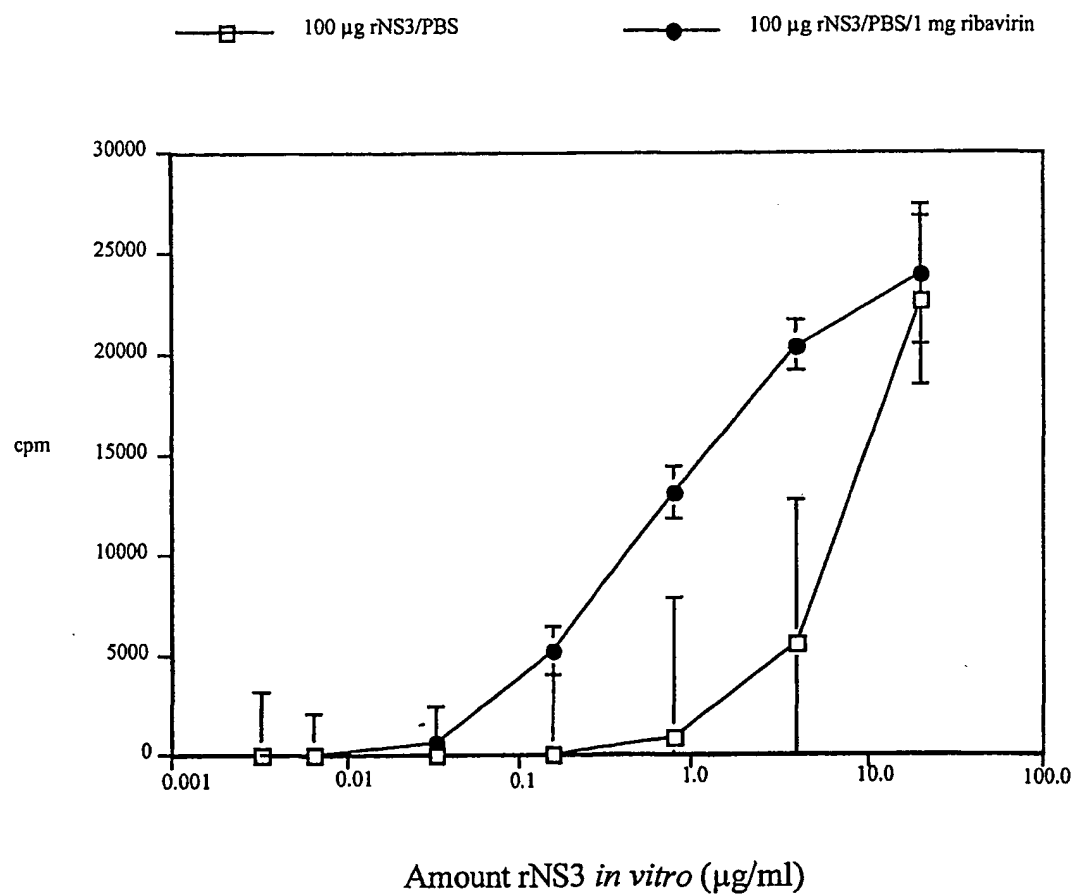
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FIGURE 2



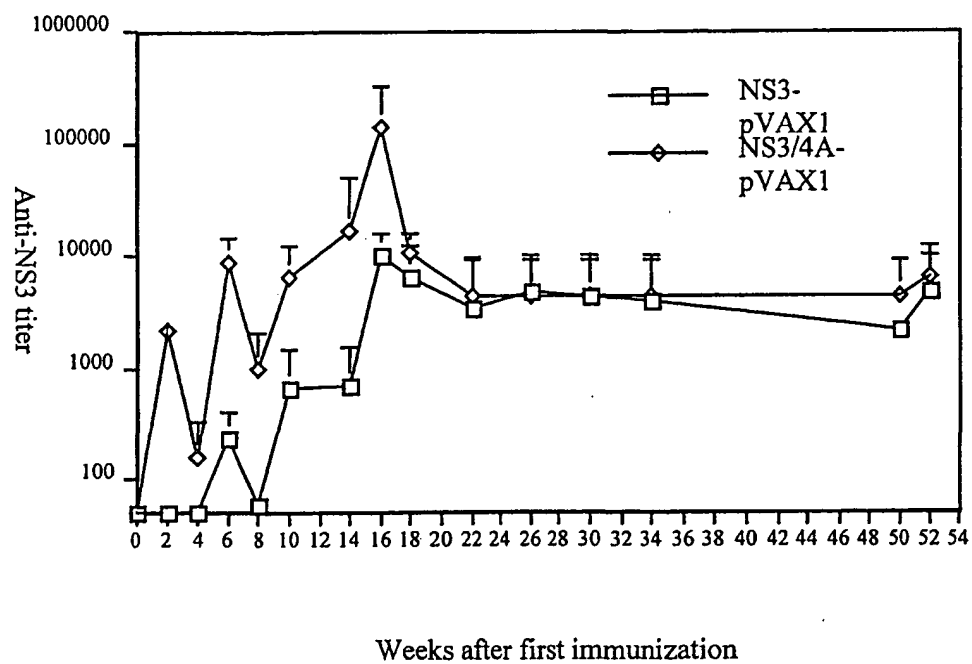
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FIGURE 3



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FIGURE 4



5/5

FIG. 5A

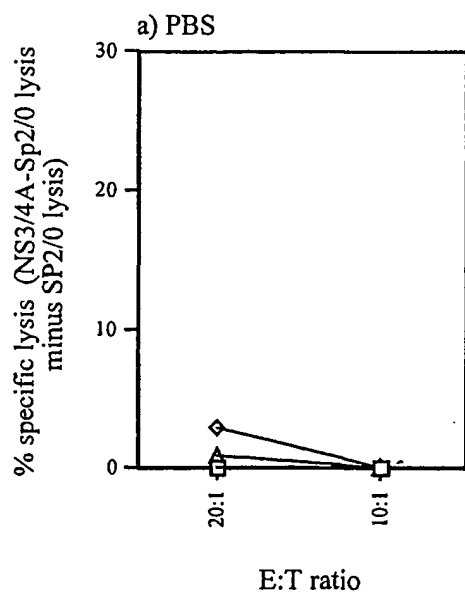
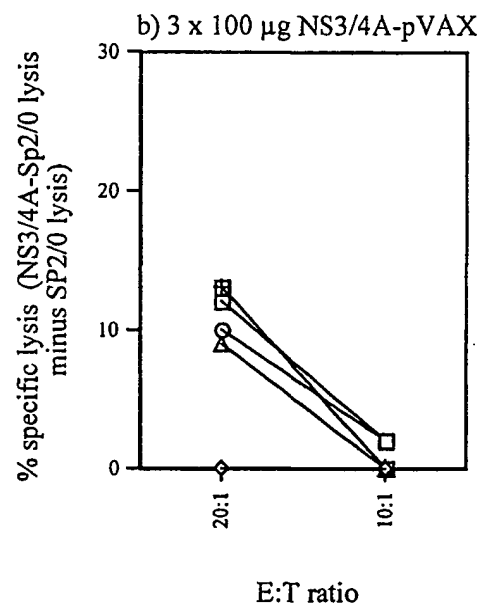


FIG. 5B



SEQUENCE LISTING

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Matti SALLBERG

Catharina HULTGREN

<120> VACCINES CONTAINING RIBAVIRIN AND
METHODS OF USE THEREOF

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<151> 2000-11-03

<150> US 60/229,175

<151> 2000-08-29

<150> US 60/225,767

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<212> PRT

<213> Artificial Sequence

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<223> Hepatitis C virus sequence

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 Asn Ser Ser Val Val Tyr Glu Ala Ala Asp Ala Ile Leu His Thr Pro
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- 3 -

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 Asp Leu Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu Tyr Val Gly
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 Thr Ala Gly Leu Val Gly Leu Leu Thr Pro Gly Ala Lys Gln Asn Ile
 20 25 30
 Gln Leu Ile Asn Thr Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu
 35 40 45
 Asn Cys Asn Glu Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr
 50 55 60
 Gln His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys
 65 70 75 80
 Arg Arg Leu Thr Asp Phe Ala Gln Gly Trp Gly Pro Ile Ser Tyr Ala
 85 90 95
 Asn Gly Ser Gly Leu Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro
 100 105 110
 Arg Pro Cys Gly Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr
 115 120 125
 Cys Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly
 130 135 140
 Ala Pro Thr Tyr Ser Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu
 145 150 155 160
 Asn Asn Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met
 165 170 175
 Asn Ser Thr Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile
 180 185 190
 Gly Gly Val Gly Asn Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg
 195 200 205
 Lys Tyr Pro Glu Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Arg Ile
 210 215 220
 Thr Pro Arg Cys Met Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro
 225 230 235 240
 Cys Thr Ile Asn Tyr Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly
 245 250 255
 Val Glu His Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg
 260 265 270
 Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu
 275 280 285
 Ser Thr Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro
 290 295 300
 Ala Leu Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val
 305 310 315 320
 Gln Tyr Leu Tyr Gly Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys
 325 330 335
 Trp Glu Tyr Val Val Leu Leu Phe Leu Leu Leu Ala Asp Ala
 340 345 350

<210> 5

<211> 315

<212> PRT

<213> Artificial Sequence

<220>

<223> Hepatitis C virus NS2 protein sequence

<400> 5

```

Arg Val Cys Ser Cys Leu Trp Met Met Leu Leu Ile Ser Gln Ala Glu
 1          5          10          15
Ala Ala Leu Glu Asn Leu Val Ile Leu Asn Ala Ala Ser Leu Ala Gly
 20          25          30
Thr His Gly Leu Val Ser Phe Leu Val Phe Phe Cys Phe Ala Trp Tyr
 35          40          45
Leu Lys Gly Arg Trp Val Pro Gly Ala Val Tyr Ala Leu Tyr Gly Met
 50          55          60
Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu Pro Gln Arg Ala Tyr Ala
 65          70          75          80
Leu Asp Thr Glu Val Ala Ala Ser Cys Gly Gly Val Val Leu Val Gly
 85          90          95
Leu Met Ala Leu Thr Leu Ser Pro Tyr Tyr Lys Arg Tyr Ile Ser Trp
100          105          110
Cys Met Trp Trp Leu Gln Tyr Phe Leu Thr Arg Val Glu Ala Gln Leu
115          120          125
His Val Trp Val Pro Pro Leu Asn Val Arg Gly Gly Arg Asp Ala Val
130          135          140
Ile Leu Leu Thr Cys Val Val His Pro Ala Leu Val Phe Asp Ile Thr
145          150          155          160
Lys Leu Leu Leu Ala Ile Phe Gly Pro Leu Trp Ile Leu Gln Ala Ser
165          170          175
Leu Leu Lys Val Pro Tyr Phe Val Arg Val Gln Gly Leu Leu Arg Ile
180          185          190
Cys Ala Leu Ala Arg Lys Ile Ala Gly Gly His Tyr Val Gln Met Ala
195          200          205
Ile Ile Lys Leu Gly Ala Leu Thr Gly Thr Cys Val Tyr Asn His Leu
210          215          220
Ala Pro Leu Arg Asp Trp Ala His Asn Gly Leu Arg Asp Leu Ala Val
225          230          235          240
Ala Val Glu Pro Val Val Phe Ser Arg Met Glu Thr Lys Leu Ile Thr
245          250          255
Trp Gly Ala Asp Thr Ala Ala Cys Gly Asp Ile Ile Asn Gly Leu Pro
260          265          270
Val Ser Ala Arg Arg Gly Gln Glu Ile Leu Leu Gly Pro Ala Asp Gly
275          280          285
Met Val Ser Lys Gly Trp Arg Leu Leu Ala Pro Ile Thr Ala Tyr Ala
290          295          300
Gln Gln Thr Arg Gly Leu Leu Gly Cys Ile Ile
305          310          315

```

<210> 6

<211> 613

<212> PRT

<213> Artificial Sequence

<220>

<223> Hepatitis C virus NS3 protein sequence

<400> 6

```

Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly Glu Val Gln
 1          5          10          15
Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala Thr Cys Ile Asn Gly

```

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```

Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala Gly
465                               470                               475                               480
Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val Arg Leu Arg
                               485                               490                               495
Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Gly
                               500                               505                               510
Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe
                               515                               520                               525
Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Phe Pro Tyr Leu Val Ala
                               530                               535                               540
Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser Trp
545                               550                               555                               560
Asp Gln Met Arg Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly
                               565                               570                               575
Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Val Thr
                               580                               585                               590
Leu Thr His Pro Ile Thr Lys Tyr Ile Met Thr Cys Met Ser Ala Asp
                               595                               600                               605
Leu Glu Val Val Thr
610

```

<210> 7
 <211> 54
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hepatitis C virus NS4A protein sequence

```

<400> 7
Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala Tyr
1                               5                               10                               15
Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val Leu Ser
                               20                               25                               30
Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Gln Glu Phe
                               35                               40                               45
Asp Glu Met Glu Glu Cys
50

```

<210> 8
 <211> 260
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hepatitis C virus NS4B protein sequence

```

<400> 8
Ser Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln
1                               5                               10                               15
Phe Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser Arg His Ala
                               20                               25                               30
Glu Val Ile Thr Pro Ala Val Gln Thr Asn Trp Gln Lys Leu Glu Val

```

```

      35      40      45
Phe Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu
  50      55      60
Ala Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met
  65      70      75      80
Ala Phe Thr Ala Ala Val Thr Ser Pro Leu Thr Thr Gly Gln Thr Leu
      85      90      95
Leu Phe Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Ala Pro
      100      105      110
Gly Ala Ala Thr Ala Phe Val Gly Ala Gly Leu Ala Gly Ala Ala Leu
      115      120      125
Asp Ser Val Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr
      130      135      140
Gly Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly
      145      150      155      160
Glu Val Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu
      165      170      175
Ser Pro Gly Ala Leu Ala Val Gly Val Val Phe Ala Ser Ile Leu Arg
      180      185      190
Arg Arg Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu
      195      200      205
Ile Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val
      210      215      220
Pro Glu Ser Asp Ala Ala Ala Arg Val Thr Ala Ile Leu Ser Ser Leu
      225      230      235      240
Thr Val Thr Gln Leu Leu Arg Arg Leu His Gln Trp Ile Ser Ser Glu
      245      250      255
Cys Thr Thr Pro
      260

```

<210> 9

<211> 1040

<212> PRT

<213> Artificial Sequence

<220>

<223> Hepatitis C virus NS5A/B protein sequence

<400> 9

```

Cys Ser Gly Ser Trp Leu Arg Asp Ile Trp Asp Trp Ile Cys Glu Val
  1      5      10      15
Leu Ser Asp Phe Lys Thr Trp Leu Lys Ala Lys Leu Met Pro Gln Leu
      20      25      30
Pro Gly Ile Pro Phe Val Ser Cys Gln Arg Gly Tyr Arg Gly Val Trp
      35      40      45
Arg Gly Asp Gly Ile Met His Thr Arg Cys His Cys Gly Ala Glu Ile
      50      55      60
Thr Gly His Val Lys Asn Gly Thr Met Arg Ile Val Gly Pro Arg Thr
      65      70      75      80
Cys Lys Asn Met Trp Ser Gly Thr Phe Phe Ile Asn Ala Tyr Thr Thr
      85      90      95
Gly Pro Cys Thr Pro Leu Pro Ala Pro Asn Tyr Lys Phe Ala Leu Trp
      100      105      110
Arg Val Ser Ala Glu Glu Tyr Val Glu Ile Arg Arg Val Gly Asp Phe

```

- 15 -

- 16 -

```

          995          1000          1005
Ile Tyr His Ser Val Ser His Ala Arg Pro Arg Trp Phe Trp Phe Cys
    1010          1015          1020
Leu Leu Leu Leu Ala Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg
1025          1030          1035          1040

```

<210> 10
 <211> 226
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hepatitis B virus S antigen (HBsAg) sequence

```

<400> 10
Met Glu Asn Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu Val Leu Gln
 1          5          10          15
Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu
    20          25          30
Asp Ser Trp Trp Thr Ser Leu Asp Phe Leu Gly Gly Thr Thr Val Cys
    35          40          45
Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser
    50          55          60
Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe
65          70          75          80
Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val
    85          90          95
Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly
    100          105          110
Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala
    115          120          125
Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp
    130          135          140
Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys
145          150          155          160
Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu
    165          170          175
Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu
    180          185          190
Ser Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile
    195          200          205
Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val
    210          215          220
Tyr Ile
225

```

<210> 11
 <211> 212
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hepatitis B virus C antigen and e antigen

(HBcAg/HBeAg) sequence

<400> 11

```

Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Ser Cys Pro Thr
 1           5           10           15
Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Gly Met Asp Ile
          20           25           30
Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu Ser Phe Leu
          35           40           45
Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp Thr Ala Ser
          50           55           60
Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys Ser Pro His
65           70           75           80
His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu Leu Met Thr
          85           90           95
Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala Ser Arg Asp
          100          105          110
Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys Phe Arg Gln
          115          120          125
Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
          130          135          140
Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala
145           150           155           160
Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu Thr Thr
          165           170           175
Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro
          180          185          190
Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg
          195          200          205
Glu Ser Gln Cys
          210

```

<210> 12

<211> 2227

<212> PRT

<213> Artificial Sequence

<220>

<223> Hepatitis A virus sequence

<400> 12

```

Met Asn Met Ser Lys Gln Gly Ile Phe Gln Thr Val Gly Ser Gly Leu
 1           5           10           15
Asp His Ile Leu Ser Leu Ala Asp Ile Glu Glu Glu Gln Met Ile Gln
          20           25           30
Ser Val Asp Arg Thr Ala Val Thr Gly Ala Ser Tyr Phe Thr Ser Val
          35           40           45
Asp Gln Ser Ser Val His Thr Ala Glu Val Gly Ser His Gln Ile Glu
          50           55           60
Pro Leu Lys Thr Ser Val Asp Lys Pro Gly Ser Lys Lys Thr Gln Gly
65           70           75           80
Glu Lys Phe Phe Leu Ile His Ser Ala Asp Trp Leu Thr Thr His Ala
          85           90           95
Leu Phe His Glu Val Ala Lys Leu Asp Val Val Lys Leu Leu Tyr Asn

```

----- Glu-Glu-Phe-Ser-Thr-Thr-Val Ser-Thr-Glu-Gln Asn Val Pro Asp Pro

Gln Gln Leu Asn Asp Pro Val Leu Ala Lys Lys Val Pro Glu Thr Phe
 545 550 555 560
 Pro Glu Leu Lys Pro Gly Glu Ser Arg His Thr Ser Asp His Met Ser
 565 570 575
 Ile Tyr Lys Phe Met Gly Arg Ser His Phe Leu Cys Thr Phe Thr Phe
 580 585 590
 Asn Ser Asn Asn Lys Glu Tyr Thr Phe Pro Ile Thr Leu Ser Ser Thr
 595 600 605
 Ser Asn Pro Pro His Gly Leu Pro Ser Thr Leu Arg Trp Phe Phe Asn
 610 615 620
 Leu Phe Gln Leu Tyr Arg Gly Pro Leu Asp Leu Thr Ile Ile Ile Thr
 625 630 635 640
 Gly Ala Thr Asp Val Asp Gly Met Ala Trp Phe Thr Pro Val Gly Leu
 645 650 655
 Ala Val Asp Pro Trp Val Glu Lys Glu Ser Ala Leu Ser Ile Asp Tyr
 660 665 670
 Lys Thr Ala Leu Gly Ala Val Arg Phe Asn Thr Arg Arg Thr Gly Asn
 675 680 685
 Ile Gln Ile Arg Leu Pro Trp Tyr Ser Tyr Leu Tyr Ala Val Ser Gly
 690 695 700
 Ala Leu Asp Gly Leu Gly Asp Lys Thr Asp Ser Thr Phe Gly Leu Phe
 705 710 715 720
 Leu Phe Glu Ile Ala Asn Tyr Asn His Ser Asp Glu Tyr Leu Ser Phe
 725 730 735
 Ser Cys Tyr Leu Ser Val Thr Glu Gln Ser Glu Phe Tyr Phe Pro Arg
 740 745 750
 Ala Pro Leu Asn Ser Asn Ala Met Leu Ser Thr Glu Ser Met Met Ser
 755 760 765
 Arg Ile Ala Ala Gly Asp Leu Glu Ser Ser Val Asp Asp Pro Arg Ser
 770 775 780
 Glu Glu Asp Arg Arg Phe Glu Ser His Ile Glu Cys Arg Lys Pro Tyr
 785 790 795 800
 Lys Glu Leu Arg Leu Glu Val Gly Lys Gln Arg Leu Lys Tyr Ala Gln
 805 810 815
 Glu Glu Leu Ser Asn Glu Val Leu Pro Pro Pro Arg Lys Met Lys Gly
 820 825 830
 Leu Phe Ser Gln Ala Lys Ile Ser Leu Phe Tyr Thr Glu Glu His Glu
 835 840 845
 Ile Met Lys Phe Ser Trp Arg Gly Val Thr Ala Asp Thr Arg Ala Leu
 850 855 860
 Arg Arg Phe Gly Phe Ser Leu Ala Ala Gly Arg Ser Val Trp Thr Leu
 865 870 875 880
 Glu Met Asp Ala Gly Val Leu Thr Gly Arg Leu Ile Arg Leu Asn Asp
 885 890 895
 Glu Lys Trp Thr Glu Met Lys Asp Asp Lys Ile Val Ser Leu Ile Glu
 900 905 910
 Lys Phe Thr Ser Asn Lys Tyr Trp Ser Lys Val Asn Phe Pro His Gly
 915 920 925
 Met Leu Asp Leu Glu Glu Ile Ala Ala Asn Ser Lys Asp Phe Pro Asn
 930 935 940
 Met Ser Glu Thr Asp Leu Cys Phe Leu Leu His Trp Leu Asn Pro Lys
 945 950 955 960
 Lys Ile Asn Leu Ala Asp Arg Met Leu Gly Leu Ser Gly Val Gln Glu
 965 970 975
 Ile Lys Glu Gln Gly Val Gly Leu Ile Ala Glu Cys Arg Thr Phe Leu

980				985				990							
Asp	Ser	Ile	Ala	Gly	Thr	Leu	Lys	Ser	Met	Met	Phe	Gly	Phe	His	His
995				1000				1005							
Ser	Val	Thr	Val	Glu	Ile	Ile	Asn	Thr	Val	Leu	Cys	Phe	Val	Lys	Ser
1010				1015				1020							
Gly	Ile	Leu	Leu	Tyr	Val	Ile	Gln	Gln	Leu	Asn	Gln	Asp	Glu	His	Ser
1025				1030				1035				1040			
His	Ile	Ile	Gly	Leu	Leu	Arg	Val	Met	Asn	Tyr	Ala	Asp	Ile	Gly	Cys
1045				1050				1055							
Ser	Val	Ile	Ser	Cys	Gly	Lys	Val	Phe	Ser	Lys	Met	Leu	Glu	Thr	Val
1060				1065				1070							
Phe	Asn	Trp	Gln	Met	Asp	Ser	Arg	Met	Met	Glu	Leu	Arg	Thr	Gln	Ser
1075				1080				1085							
Phe	Ser	Asn	Trp	Leu	Arg	Asp	Ile	Cys	Ser	Gly	Ile	Thr	Ile	Phe	Lys
1090				1095				1100							
Ser	Phe	Lys	Asp	Ala	Ile	Tyr	Trp	Leu	Tyr	Thr	Lys	Leu	Lys	Asp	Phe
1105				1110				1115				1120			
Tyr	Glu	Val	Asn	Tyr	Gly	Lys	Lys	Lys	Asp	Ile	Leu	Asn	Ile	Leu	Lys
1125				1130				1135							
Asp	Asn	Gln	Gln	Lys	Ile	Glu	Lys	Ala	Ile	Glu	Glu	Ala	Asp	Asn	Phe
1140				1145				1150							
Cys	Ile	Leu	Gln	Ile	Gln	Asp	Val	Glu	Lys	Phe	Asp	Gln	Tyr	Gln	Lys
1155				1160				1165							
Gly	Val	Asp	Leu	Ile	Gln	Lys	Leu	Arg	Thr	Val	His	Ser	Met	Ala	Gln
1170				1175				1180							
Val	Asp	Pro	Asn	Leu	Gly	Val	His	Leu	Ser	Pro	Leu	Arg	Asp	Cys	Ile
1185				1190				1195				1200			
Ala	Arg	Val	His	Gln	Lys	Leu	Lys	Asn	Leu	Gly	Ser	Ile	Asn	Gln	Ala
1205				1210				1215							
Met	Val	Thr	Arg	Cys	Glu	Pro	Val	Val	Cys	Tyr	Leu	Tyr	Gly	Lys	Arg
1220				1225				1230							
Gly	Gly	Gly	Lys	Ser	Leu	Thr	Ser	Ile	Ala	Leu	Ala	Thr	Lys	Ile	Cys
1235				1240				1245							
Lys	His	Tyr	Gly	Val	Glu	Pro	Glu	Lys	Asn	Ile	Tyr	Thr	Lys	Pro	Val
1250				1255				1260							
Ala	Ser	Asp	Tyr	Trp	Asp	Gly	Tyr	Ser	Gly	Gln	Leu	Val	Cys	Ile	Ile
1265				1270				1275				1280			
Asp	Asp	Ile	Gly	Gln	Asn	Thr	Thr	Asp	Glu	Asp	Trp	Ser	Asp	Phe	Cys
1285				1290				1295							
Gln	Leu	Val	Ser	Gly	Cys	Pro	Met	Arg	Leu	Asn	Met	Ala	Ser	Leu	Glu
1300				1305				1310							
Glu	Lys	Gly	Arg	His	Phe	Ser	Ser	Pro	Phe	Ile	Ile	Ala	Thr	Ser	Asn
1315				1320				1325							
Trp	Ser	Asn	Pro	Ser	Pro	Lys	Thr	Val	Tyr	Val	Lys	Glu	Ala	Ile	Asp
1330				1335				1340							
Arg	Arg	Leu	His	Phe	Lys	Val	Glu	Val	Lys	Pro	Ala	Ser	Phe	Phe	Lys
1345				1350				1355				1360			
Asn	Pro	His	Asn	Asp	Met	Leu	Asn	Val	Asn	Leu	Ala	Lys	Thr	Asn	Asp
1365				1370				1375							
Ala	Ile	Lys	Asp	Met	Ser	Cys	Val	Asp	Leu	Ile	Met	Asp	Gly	His	Asn
1380				1385				1390							
Ile	Ser	Leu	Met	Asp	Leu	Leu	Ser	Ser	Leu	Val	Met	Thr	Val	Glu	Ile
1395				1400				1405							
Arg	Lys	Gln	Asn	Met	Ser	Glu	Phe	Met	Glu	Leu	Trp	Ser	Gln	Gly	Ile
1410				1415				1420							

Ser Asp Asp Asp Asn Asp Ser Ala Val Ala Glu Phe Phe Gln Ser Phe
 1425 1430 1435 1440
 Pro Ser Gly Glu Pro Ser Asn Trp Lys Leu Ser Ser Phe Phe Gln Ser
 1445 1450 1455
 Val Thr Asn His Lys Trp Val Ala Val Gly Ala Ala Val Gly Ile Leu
 1460 1465 1470
 Gly Val Leu Val Gly Gly Trp Phe Val Tyr Lys His Phe Ser Arg Lys
 1475 1480 1485
 Glu Glu Glu Pro Ile Pro Ala Glu Gly Val Tyr His Gly Val Thr Lys
 1490 1495 1500
 Pro Lys Gln Val Ile Lys Leu Asp Ala Asp Pro Val Glu Ser Gln Ser
 1505 1510 1515 1520
 Thr Leu Glu Ile Ala Gly Leu Val Arg Lys Asn Leu Val Gln Phe Gly
 1525 1530 1535
 Val Gly Glu Lys Asn Gly Cys Val Arg Trp Val Met Asn Ala Leu Gly
 1540 1545 1550
 Val Lys Asp Asp Trp Leu Leu Val Pro Ser His Ala Tyr Lys Phe Glu
 1555 1560 1565
 Lys Asp Tyr Glu Met Met Glu Phe Tyr Phe Asn Arg Gly Gly Thr Tyr
 1570 1575 1580
 Tyr Ser Ile Ser Ala Gly Asn Val Val Ile Gln Ser Leu Asp Val Gly
 1585 1590 1595 1600
 Phe Gln Asp Val Val Leu Met Lys Val Pro Thr Ile Pro Lys Phe Arg
 1605 1610 1615
 Asp Ile Thr Gln His Phe Ile Lys Lys Gly Asp Val Pro Arg Ala Leu
 1620 1625 1630
 Asn Arg Leu Ala Thr Leu Val Thr Thr Val Asn Gly Thr Pro Met Leu
 1635 1640 1645
 Ile Ser Glu Gly Pro Leu Lys Met Glu Glu Lys Ala Thr Tyr Val His
 1650 1655 1660
 Lys Lys Asn Asp Gly Thr Thr Val Asp Leu Thr Val Asp Gln Ala Trp
 1665 1670 1675 1680
 Arg Gly Lys Gly Glu Gly Leu Pro Gly Met Cys Gly Gly Ala Leu Val
 1685 1690 1695
 Ser Ser Asn Gln Ser Ile Gln Asn Ala Ile Leu Gly Ile His Val Ala
 1700 1705 1710
 Gly Gly Asn Ser Ile Leu Val Ala Lys Leu Val Thr Gln Glu Met Phe
 1715 1720 1725
 Gln Asn Ile Asp Lys Lys Ile Glu Ser Gln Arg Ile Met Lys Val Glu
 1730 1735 1740
 Phe Thr Gln Cys Ser Met Asn Val Val Ser Lys Thr Leu Phe Arg Lys
 1745 1750 1755 1760
 Ser Pro Ile His His His Ile Asp Lys Thr Met Ile Asn Phe Pro Ala
 1765 1770 1775
 Ala Met Pro Phe Ser Lys Ala Glu Ile Asp Pro Met Ala Met Met Leu
 1780 1785 1790
 Ser Lys Tyr Ser Leu Pro Ile Val Glu Glu Pro Glu Asp Tyr Lys Glu
 1795 1800 1805
 Ala Ser Val Phe Tyr Gln Asn Lys Ile Val Gly Lys Thr Gln Leu Val
 1810 1815 1820
 Asp Asp Phe Leu Asp Leu Asp Met Ala Ile Thr Gly Ala Pro Gly Ile
 1825 1830 1835 1840
 Asp Ala Ile Asn Met Asp Ser Ser Pro Gly Phe Pro Tyr Val Gln Glu
 1845 1850 1855
 Lys Leu Thr Lys Arg Asp Leu Ile Trp Leu Asp Glu Asn Gly Leu Leu

	1860		1865		1870
Leu Gly Val His Pro Arg Leu Ala Gln Arg Ile Leu Phe Asn Thr Val					
1875		1880		1885	
Met Met Glu Asn Cys Ser Asp Leu Asp Val Val Phe Thr Thr Cys Pro					
1890		1895		1900	
Lys Asp Glu Leu Arg Pro Leu Glu Lys Val Leu Glu Ser Lys Thr Arg					
1905		1910		1915	1920
Ala Ile Asp Ala Cys Pro Leu Asp Tyr Thr Ile Leu Cys Arg Met Tyr					
	1925		1930		1935
Trp Gly Pro Ala Ile Ser Tyr Phe His Leu Asn Pro Gly Phe His Thr					
	1940		1945		1950
Gly Val Ala Ile Gly Ile Asp Pro Asp Arg Gln Trp Asp Glu Leu Phe					
	1955		1960		1965
Lys Thr Met Ile Arg Phe Gly Asp Val Gly Leu Asp Leu Asp Phe Ser					
	1970		1975		1980
Ala Phe Asp Ala Ser Leu Ser Pro Phe Met Ile Arg Glu Ala Gly Arg					
1985		1990		1995	2000
Ile Met Ser Glu Leu Ser Gly Thr Pro Ser His Phe Gly Thr Ala Leu					
	2005		2010		2015
Ile Asn Thr Ile Ile Tyr Ser Lys His Leu Leu Tyr Asn Cys Cys Tyr					
	2020		2025		2030
His Val Cys Gly Ser Met Pro Ser Gly Ser Pro Cys Thr Ala Leu Leu					
	2035		2040		2045
Asn Ser Ile Ile Asn Asn Ile Asn Leu Tyr Tyr Val Phe Ser Lys Ile					
	2050		2055		2060
Phe Gly Lys Ser Pro Val Phe Phe Cys Gln Ala Leu Arg Ile Leu Cys					
2065		2070		2075	2080
Tyr Gly Asp Asp Val Leu Ile Val Phe Ser Arg Asp Val Gln Ile Asp					
	2085		2090		2095
Asn Leu Asp Leu Ile Gly Gln Lys Ile Val Asp Glu Phe Lys Lys Leu					
	2100		2105		2110
Gly Met Thr Ala Thr Ser Ala Asp Lys Asn Val Pro Gln Leu Lys Pro					
	2115		2120		2125
Val Ser Glu Leu Thr Phe Leu Lys Arg Ser Phe Asn Leu Val Glu Asp					
	2130		2135		2140
Arg Ile Arg Pro Ala Ile Ser Glu Lys Thr Ile Trp Ser Leu Met Ala					
2145		2150		2155	2160
Trp Gln Arg Ser Asn Ala Glu Phe Glu Gln Asn Leu Glu Asn Ala Gln					
	2165		2170		2175
Trp Phe Ala Phe Met His Gly Tyr Glu Phe Tyr Gln Lys Phe Tyr Tyr					
	2180		2185		2190
Phe Val Gln Ser Cys Leu Glu Lys Glu Met Ile Glu Tyr Arg Leu Lys					
	2195		2200		2205
Ser Tyr Asp Trp Trp Arg Met Arg Phe Tyr Asp Gln Cys Phe Ile Cys					
	2210		2215		2220
Asp Leu Ser					
2225					

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<223> Hepatitis C virus sequence

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<223> Hepatitis C virus NS3/4A, coding region

<400> 16

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<223> Hepatitis C virus NS3/4A peptide

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Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
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65             70             75             80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
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Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
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Asp Val Ile Pro Val Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
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Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
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Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
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Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
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 Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
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 Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
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<212> DNA

<213> Artificial Sequence

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<223> cloning oligonucleotide

<400> 18

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<210> 20

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<220>
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<210> 26
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<220>
 <223> Mutant Hepatitis C virus NS3/4A peptide

<400> 26

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<210> 27

<211> 25

<212> PRT

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<223> Mutant Hepatitis C virus NS3/4A peptide

<400> 27

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<211> 12

<212> PRT

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<223> Hepatitis C virus NS3/4A peptide

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<210> 29

<211> 632

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<213> Hepatitis C virus NS3 peptideArtificial Sequence

<220>

<223> Hepatitis C virus NS3 peptide

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Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
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 245 250 255
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 260 265 270
 Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
 275 280 285
 Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile
 290 295 300
 Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val
 305 310 315 320
 Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
 325 330 335
 Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly
 340 345 350
 Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe
 355 360 365
 Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala
 370 375 380
 Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
 385 390 395 400
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
 405 410 415
 Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
 420 425 430
 Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
 435 440 445
 Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly
 450 455 460
 Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly
 465 470 475 480
 Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
 485 490 495
 Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val
 500 505 510
 Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp

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      515      520      525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
      530      535      540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545      550      555      560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
      565      570      575
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
      580      585      590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
      595      600      605
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
      610      615      620
Ser Ala Asp Leu Glu Val Val Thr
625      630

```

<210> 30

<211> 54

<212> PRT

<213> Artificial Sequence

<220>

<223> Hepatitis C virus NS4A peptide

<400> 30

```

Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala Tyr
 1      5      10      15
Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val Leu Ser
      20      25      30
Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Arg Glu Phe
      35      40      45
Asp Glu Met Glu Glu Cys
50

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<210> 31

<211> 686

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Hepatitis C virus NS3/4A

<400> 31

```

Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1      5      10      15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
      20      25      30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
      35      40      45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
      50      55      60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65      70      75      80

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Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
 85 90 95
 Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
 100 105 110
 Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
 115 120 125
 Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
 130 135 140
 Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
 145 150 155 160
 Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
 165 170 175
 Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro
 180 185 190
 Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly
 195 200 205
 Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
 210 215 220
 Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly
 225 230 235 240
 Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
 245 250 255
 Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
 260 265 270
 Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
 275 280 285
 Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile
 290 295 300
 Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val
 305 310 315 320
 Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
 325 330 335
 Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly
 340 345 350
 Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe
 355 360 365
 Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala
 370 375 380
 Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
 385 390 395 400
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
 405 410 415
 Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
 420 425 430
 Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
 435 440 445
 Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly
 450 455 460
 Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly
 465 470 475 480
 Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
 485 490 495
 Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val
 500 505 510
 Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp

```

      515              520 -              525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
      530              535              540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545              550              555              560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
      565              570              575
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
      580              585              590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
      595              600              605
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
      610              615              620
Ser Ala Asp Leu Glu Val Val Thr Gly Thr Trp Val Leu Val Gly Gly
625              630              635              640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
      645              650              655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
      660              665              670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
      675              680              685

```

<210> 32

<211> 686

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Hepatitis C virus NS3/4A

<400> 32

```

Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
  1              5              10              15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
      20              25              30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
      35              40              45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
      50              55              60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65              70              75              80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
      85              90              95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
      100              105              110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
      115              120              125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
      130              135              140
-----
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145              150              155              160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
      165              170              175
Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro

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- 40 -

Ser Ala Asp Leu Glu Val Val Arg Gly Thr Trp Val Leu Val Gly Gly
 625 630 635 640
 Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
 645 650 655
 Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
 660 665 670
 Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
 675 680 685

<210> 33
 <211> 25
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Mutant Hepatitis C virus NS3/4A peptide

<400> 33
 Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Thr Pro
 1 5 10 15
 Thr Trp Val Leu Val Gly Gly Val Leu
 20 25

<210> 34
 <211> 25
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Mutant Hepatitis C virus NS3/4A peptide

<400> 34
 Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Arg Pro
 1 5 10 15
 Thr Trp Val Leu Val Gly Gly Val Leu
 20 25

<210> 35
 <211> 25
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Mutant Hepatitis C virus NS3/4A peptide

<400> 35
 Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Arg Pro
 1 5 10 15
 Ala Trp Val Leu Val Gly Gly Val Leu
 20 25

<210> 36
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutant Hepatitis C virus, NS3/4A peptide

<400> 36
Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Cys Ser
1 5 10 15
Thr Trp Val Leu Val Gly Gly Val Leu
20 25

<210> 37
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutant Hepatitis C virus NS3/4A peptide

<400> 37
Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Cys Cys Ser
1 5 10 15
Thr Trp Val Leu Val Gly Gly Val Leu
20 25

<210> 38
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutant Hepatitis C virus NS3/4A peptide

<400> 38
Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Ser Ser Ser
1 5 10 15
Thr Trp Val Leu Val Gly Gly Val Leu
20 25

<210> 39
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutant Hepatitis C virus NS3/4A peptide

<400> 39
Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Ser Ser Ser Ser Cys Ser

1	5	10	15
Thr Trp Val Leu Val Gly Gly Val Leu			
20	25		

<210> 40
 <211> 25
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Mutant Hepatitis C virus NS3/4A peptide

<400> 40			
Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Val Val Val Val Thr Ser			
1	5	10	15
Thr Trp Val Leu Val Gly Gly Val Leu			
20	25		

<210> 41
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hepatitis C virus NS5 peptide

<400> 41			
Ala Ser Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly			
1	5	10	15

<210> 42
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Mutant Hepatitis C virus NS5A/B peptide

<400> 42			
Ser Ser Glu Asp Val Val Cys Cys Ser Met Trp Val Leu Val Gly Gly			
1	5	10	15
Val Leu			

<210> 43
 <211> 686
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Mutant Hepatitis C virus NS3/4A

<400> 43

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Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1           5           10           15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
          20           25           30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
          35           40           45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
          50           55           60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65           70           75           80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
          85           90           95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
          100          105          110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
          115          120          125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
          130          135          140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145           150           155           160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
          165          170          175
Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro
          180          185          190
Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly
          195          200          205
Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
          210          215          220
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly
225           230           235           240
Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
          245          250          255
Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
          260          265          270
Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
          275          280          285
Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile
          290          295          300
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val
305           310           315           320
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
          325          330          335
Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly
          340          345          350
Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe
          355          360          365
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala
          370          375          380
Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
385           390           395           400
Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
          405          410          415
Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys

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      420      425      430
Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
      435      440      445
Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly
      450      455      460
Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly
465      470      475      480
Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
      485      490      495
Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val
      500      505      510
Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
      515      520      525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
      530      535      540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545      550      555      560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
      565      570      575
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
      580      585      590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
      595      600      605
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
      610      615      620
Ser Ala Asp Leu Glu Val Val Thr Pro Thr Trp Val Leu Val Gly Gly
625      630      635      640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
      645      650      655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
      660      665      670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
      675      680      685

```

<210> 44

<211> 686

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Hepatitis C virus NS3/4A

<400> 44

```

Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
  1      5      10      15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
      20      25      30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
      35      40      45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
      50      55      60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65      70      75      80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr

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85										90					95							
Pro	Cys	Thr	Cys	Gly	Ser	Ser	Asp	Leu	Tyr	Leu	Val	Thr	Arg	His	Ala							
			100					105					110									
Asp	Val	Ile	Pro	Val	Arg	Arg	Arg	Gly	Asp	Gly	Arg	Gly	Ser	Leu	Leu							
		115					120					125										
Ser	Pro	Arg	Pro	Ile	Ser	Tyr	Leu	Lys	Gly	Ser	Ser	Gly	Gly	Pro	Leu							
	130					135					140											
Leu	Cys	Pro	Ala	Gly	His	Ala	Val	Gly	Ile	Phe	Arg	Ala	Ala	Val	Cys							
145					150					155					160							
Thr	Arg	Gly	Val	Ala	Lys	Ala	Val	Asp	Phe	Ile	Pro	Val	Glu	Ser	Leu							
			165					170						175								
Glu	Thr	Thr	Met	Arg	Ser	Pro	Val	Phe	Ser	Asp	Asn	Ser	Ser	Pro	Pro							
			180					185					190									
Ala	Val	Pro	Gln	Ser	Tyr	Gln	Val	Ala	His	Leu	His	Ala	Pro	Thr	Gly							
	195					200						205										
Ser	Gly	Lys	Ser	Thr	Lys	Val	Pro	Ala	Ala	Tyr	Ala	Ala	Gln	Gly	Tyr							
	210					215					220											
Lys	Val	Leu	Val	Leu	Asn	Pro	Ser	Val	Ala	Ala	Thr	Met	Gly	Phe	Gly							
225					230					235					240							
Ala	Tyr	Met	Ser	Lys	Ala	His	Gly	Ile	Asp	Pro	Asn	Ile	Arg	Thr	Gly							
			245					250						255								
Val	Arg	Thr	Ile	Thr	Thr	Gly	Ser	Pro	Ile	Thr	Tyr	Ser	Thr	Tyr	Gly							
		260						265					270									
Lys	Phe	Leu	Ala	Asp	Gly	Gly	Cys	Ser	Gly	Gly	Ala	Tyr	Asp	Ile	Ile							
	275						280					285										
Ile	Cys	Asp	Glu	Cys	His	Ser	Thr	Asp	Ala	Thr	Ser	Ile	Leu	Gly	Ile							
	290					295					300											
Gly	Thr	Val	Leu	Asp	Gln	Ala	Glu	Thr	Ala	Gly	Ala	Arg	Leu	Thr	Val							
305					310					315					320							
Leu	Ala	Thr	Ala	Thr	Pro	Pro	Gly	Ser	Val	Thr	Val	Pro	His	Pro	Asn							
			325					330						335								
Ile	Glu	Glu	Val	Ala	Leu	Ser	Thr	Thr	Gly	Glu	Ile	Pro	Phe	Tyr	Gly							
		340						345					350									
Lys	Ala	Ile	Pro	Leu	Glu	Ala	Ile	Lys	Gly	Gly	Arg	His	Leu	Ile	Phe							
	355						360					365										
Cys	His	Ser	Lys	Lys	Lys	Cys	Asp	Glu	Leu	Ala	Ala	Lys	Leu	Val	Ala							
	370					375					380											
Leu	Gly	Val	Asn	Ala	Val	Ala	Tyr	Tyr	Arg	Gly	Leu	Asp	Val	Ser	Val							
385					390					395					400							
Ile	Pro	Thr	Ser	Gly	Asp	Val	Val	Val	Val	Ala	Thr	Asp	Ala	Leu	Met							
			405					410						415								
Thr	Gly	Phe	Thr	Gly	Asp	Phe	Asp	Ser	Val	Ile	Asp	Cys	Asn	Thr	Cys							
		420					425						430									
Val	Thr	Gln	Thr	Val	Asp	Phe	Ser	Leu	Asp	Pro	Thr	Phe	Thr	Ile	Glu							
	435						440					445										
Thr	Ile	Thr	Leu	Pro	Gln	Asp	Ala	Val	Ser	Arg	Thr	Gln	Arg	Arg	Gly							
	450					455					460											
Arg	Thr	Gly	Arg	Gly	Lys	Pro	Gly	Ile	Tyr	Arg	Phe	Val	Ala	Pro	Gly							
465					470					475					480							
Glu	Arg	Pro	Ser	Gly	Met	Phe	Asp	Ser	Ser	Val	Leu	Cys	Glu	Cys	Tyr							
			485					490						495								
Asp	Ala	Gly	Cys	Ala	Trp	Tyr	Glu	Leu	Thr	Pro	Ala	Glu	Thr	Thr	Val							
	500						505					510										
Arg	Leu	Arg	Ala	Tyr	Met	Asn	Thr	Pro	Gly	Leu	Pro	Val	Cys	Gln	Asp							
	515						520					525										

His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
 530 535 540
 Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
 545 550 555 560
 Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
 565 570 575
 Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
 580 585 590
 Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
 595 600 605
 Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
 610 615 620
 Ser Ala Asp Leu Glu Val Val Arg Pro Thr Trp Val Leu Val Gly Gly
 625 630 635 640
 Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
 645 650 655
 Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
 660 665 670
 Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
 675 680 685

<210> 45

<211> 686

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Hepatitis C virus NS3/4A

<400> 45

Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1 5 10 15
 Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
 20 25 30
 Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
 35 40 45
 Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
 50 55 60
 Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
 65 70 75 80
 Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
 85 90 95
 Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
 100 105 110
 Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
 115 120 125
 Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
 130 135 140
 Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
 145 150 155 160
 Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
 165 170 175
 Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro
 180 185 190

Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly
 195 200 205
 Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
 210 215 220
 Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly
 225 230 235 240
 Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
 245 250 255
 Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
 260 265 270
 Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
 275 280 285
 Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile
 290 295 300
 Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val
 305 310 315 320
 Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
 325 330 335
 Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly
 340 345 350
 Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe
 355 360 365
 Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala
 370 375 380
 Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
 385 390 395 400
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
 405 410 415
 Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
 420 425 430
 Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
 435 440 445
 Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly
 450 455 460
 Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly
 465 470 475 480
 Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
 485 490 495
 Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val
 500 505 510
 Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
 515 520 525
 His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
 530 535 540
 Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
 545 550 555 560
 Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
 565 570 575
 Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
 580 585 590
 Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
 595 600 605
 Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
 610 615 620
 Ser Ala Asp Leu Glu Val Val Arg Pro Ala Trp Val Leu Val Gly Gly

```

625          630          635          640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
          645          650          655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
          660          665          670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
          675          680          685

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<210> 46

<211> 686

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Hepatitis C virus NS3/4A

<400> 46

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Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1          5          10          15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
          20          25          30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
          35          40          45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
          50          55          60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
          65          70          75          80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
          85          90          95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
          100          105          110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
          115          120          125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
          130          135          140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
          145          150          155          160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
          165          170          175
Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro
          180          185          190
Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly
          195          200          205
Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
          210          215          220
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly
          225          230          235          240
Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
          245          250          255
Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
          260          265          270
Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
          275          280          285
Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile

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290	295	300
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val		
305	310	315
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn		
	325	330
Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly		
	340	345
Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe		
	355	360
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala		
	370	375
Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val		
385	390	395
Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met		
	405	410
Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys		
	420	425
Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu		
	435	440
Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly		
	450	455
Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly		
465	470	475
Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr		
	485	490
Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val		
	500	505
Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp		
	515	520
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp		
	530	535
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr		
545	550	555
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro		
	565	570
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr		
	580	585
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn		
	595	600
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met		
	610	615
Ser Ala Asp Leu Glu Val Val Cys Ser Thr Trp Val Leu Val Gly Gly		
625	630	635
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val		
	645	650
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp		
	660	665
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys		
	675	680
		685

<210> 47

<211> 686

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Hepatitis C virus NS3/4A

<400> 47

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Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1           5           10           15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
          20           25           30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
          35           40           45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
          50           55           60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65           70           75           80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
          85           90           95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
          100          105          110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
          115          120          125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
          130          135          140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145          150          155          160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
          165          170          175
Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro
          180          185          190
Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly
          195          200          205
Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
          210          215          220
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly
225          230          235          240
Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
          245          250          255
Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
          260          265          270
Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
          275          280          285
Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile
          290          295          300
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val
305          310          315          320
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
          325          330          335
Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly
          340          345          350
Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe
          355          360          365
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala
          370          375          380
Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
385          390          395          400

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Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
              405                      410                      415
Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
              420                      425                      430
Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
              435                      440                      445
Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly
              450                      455                      460
Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly
465              470                      475                      480
Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
              485                      490                      495
Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val
              500                      505                      510
Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
              515                      520                      525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
              530                      535                      540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545              550                      555                      560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
              565                      570                      575
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
              580                      585                      590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
              595                      600                      605
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
              610                      615                      620
Ser Ala Asp Leu Glu Val Cys Cys Ser Thr Trp Val Leu Val Gly Gly
625              630                      635                      640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
              645                      650                      655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
              660                      665                      670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
              675                      680                      685

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<210> 48

<211> 686

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Hepatitis C virus, NS3/4A

<400> 48

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Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
  1              5              10              15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
              20              25              30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
              35              40              45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
  50              55              60

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Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65          70          75          80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
          85          90          95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
          100          105          110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
          115          120          125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
          130          135          140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
          145          150          155          160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
          165          170          175
Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro
          180          185          190
Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly
          195          200          205
Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
          210          215          220
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly
          225          230          235          240
Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
          245          250          255
Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
          260          265          270
Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
          275          280          285
Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile
          290          295          300
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val
          305          310          315          320
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
          325          330          335
Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly
          340          345          350
Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe
          355          360          365
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala
          370          375          380
Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
          385          390          395          400
Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
          405          410          415
Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
          420          425          430
Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
          435          440          445
Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly
          450          455          460
Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly
          465          470          475          480
Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
          485          490          495
Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val

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      500      505      510
Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
      515      520      525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
      530      535      540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545      550      555      560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
      565      570      575
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
      580      585      590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
      595      600      605
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
      610      615      620
Ser Ala Asp Leu Glu Val Ser Ser Ser Thr Trp Val Leu Val Gly Gly
625      630      635      640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
      645      650      655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
      660      665      670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
      675      680      685

```

<210> 49

<211> 686

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Hepatitis C virus NS3/4A

<400> 49

```

Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1      5      10      15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
      20      25      30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
      35      40      45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
      50      55      60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65      70      75      80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
      85      90      95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
      100      105      110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
      115      120      125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
      130      135      140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145      150      155      160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu

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WO 02/13855

PCT/IB01/01808

Glu	Val	Thr	Leu	Thr	His	Pro	Val	Thr	Lys	Tyr	Ile	Met	Thr	Cys	Met
610						615					620				
Ser	Ala	Asp	Ser	Ser	Ser	Ser	Cys	Ser	Thr	Trp	Val	Leu	Val	Gly	Gly
625					630					635				640	
Val	Leu	Ala	Ala	Leu	Ala	Ala	Tyr	Cys	Leu	Ser	Thr	Gly	Cys	Val	Val
				645					650					655	
Ile	Val	Gly	Arg	Ile	Val	Leu	Ser	Gly	Lys	Pro	Ala	Ile	Ile	Pro	Asp
		660						665				670			
Arg	Glu	Val	Leu	Tyr	Arg	Glu	Phe	Asp	Glu	Met	Glu	Glu	Cys		
	675						680					685			

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